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A TEXT-BOOK OF GENERAL BACTERIOLOGY

BY

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AND IN RUSH MEDICAL COLLEGE

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TO

MY MOTHER

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PREFACE TO THE NINTH EDITION

In this edition the chapter on The Parasitic Protozoa has been entirely rewritten and several other important sections have been revised and amplified. New material has been added on the bacteriology of scarlet fever, erysipelas and rheumatic fever. The section on the bacteriology of water has been extensively altered. Through the kindness of Dr. Edward F. Francis, I am enabled to reproduce some valuable figures of Bact. tularense. I am deeply indebted for numerous suggestions to my friends and colleagues, Professor John F. Norton, Professor I. S. Falk and Professor William H. Taliaferro, and am under especial obligation to the latter for his generous assistance in bringing the chapter on protozoa abreast of current knowledge and for a number of new text figures.

Edwin O. Jordan.

Chicago, Ill.

PREFACE

This book is the outgrowth of lectures given to students in the University of Chicago during the past few years. The subject is one that the writer believes should find a place in every general scientific course. Bacteriology is chiefly of professional interest to the medical student, but the subject also bears technical relations to household administration, to agriculture, to sanitation and sanitary engineering and to various industries and technological pursuits. For the general scientific student and reader bacteriology presents certain aspects that tend to widen the outlook upon a variety of human interests.

It need hardly be said that within the compass of this work an exhaustive treatment of all sides of bacteriology is impossible. The needs of the advanced worker can be met only—and that but in part—by such monumental special treatises as the Handbuch der Pathogenen Mikroörganismen, edited by Kolle and Wassermann, and the Technische Mykologie, edited by Lafar. A general introduction to the subject, however, with some regard for perspective and with emphasis on general rather than on special questions has seemed worth attempting.

The reader who wishes to acquire greater familiarity with the subject will find some bibliographical references given as a sort of first aid to the investigator. These include references to some articles of classic or historic interest, to some giving valuable summaries or bibliographies of important subjects and to a few in fields where investigation is very active or opinions considerably at variance. No pretension to completeness is made.

The fundamental principles and methods of laboratory work are treated as fully as seems desirable in a book of this class. The tendency manifested in all the natural sciences towards the elaboration of special laboratory manuals and guides has much in its favor. A number of such guides for bacteriology are in existence, among

which may be mentioned the excellent manuals of Frost, Gorham, Heinemann, Moore and Novy, to mention only American authors. In any case a proper familiarity with laboratory methods can be gained only with the assistance of a skilled laboratory instructor possessed of individuality and resource.

I have been greatly assisted by many friends and colleagues in the preparation of this work, and to all I wish to express my cordial thanks. I am particularly indebted to Professors Ludvig Hektoen and N. McL. Harris and to Drs. P. G. Heinemann and Mary Hefferan, who have helped me in a variety of ways. Finally I would acknowledge my deep obligation to my wife, who has aided me in the preparation of the book at every point, especially in the revision of the manuscript and proof-sheets.

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General Bacteriology

CHAPTER I

INTRODUCTION

The Discovery of Bacteria.—The belief that there are living organisms too small to be seen by the unaided human eye, and that such invisible organisms play an important part in various natural phenomena, has found utterance many times since the dawn of history. Several of the philosophers of antiquity were bold enough to surmise that such organisms existed, and some writers even framed their speculations on this subject in phrases that seem like far-seeing anticipations of modern discoveries. Interesting in some degree as these speculations are, they appear to have had no influence whatever upon the course of scientific investigation and to have been let fall at random by their authors, like hundreds of similar conjectures, without any real basis in observation or experiment. The fact is that prior to the work of the Dutch microscopist, Anton van Leeuwenhoek, in the latter part of the seventeenth century, definite ocular evidence for such a belief did not exist. Leeuwenhoek (1632–1723), who was a skilled lens-maker of Delft, Holland, spent many years in examining through his microscope a great variety of natural objects, with unremitting industry if without system, and in the course of his observations chanced to come across the organisms now known as bacteria. In a letter to the Royal Society of London, dated September 14, 1683, he records in these words his observations upon some tartar scraped from the teeth and mixed with water: "I saw with wonder that my material contained many tiny animals which moved about in a most amusing fashion; the largest of these (*A*, Fig. 1) showed the liveliest and most active motion, moving through the water or saliva as a fish of prey darts through the sea; they were found everywhere, although not in large numbers. A second kind was similar to that marked *B* (Fig. 1). These sometimes spun around in a circle like a top,

and sometimes described a path like that shown in *C–D* (Fig. 1); they were present in larger numbers. A third kind could not be distinguished so clearly; now they appeared oblong, now quite round. They were so very small that they did not seem larger than the bodies marked *E*, and besides they moved so rapidly that they were continually running into one another: they looked like a swarm of gnats or flies dancing about together. I had the impression that I was looking at several thousands in a given part of the water or saliva mixed with a particle of the material from the teeth no larger than a grain of sand, even when only one part of the material was added to nine parts of water or saliva. Further, the greater part of the material consisted of an extraordinary number of rods, of widely different lengths, but of the same diameter. Some were curved, some straight, as is shown in *F*; they lay irregularly and were interlaced. Since I had previously seen living animalcules of this same kind in water, I endeavored to observe whether there was life in them, but in none did I see the smallest movement that might be taken as a sign of life." Leeuwenhoek supplemented his observations with drawings, and there is no doubt that he was the first to see bacteria and describe them accurately.

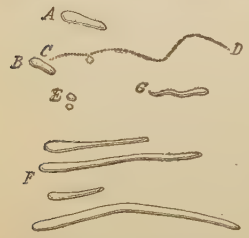


Fig. 1.—The first pictorial representation of bacteria. Leeuwenhoek, 1683 (Löffler).

The Origin of Bacteriology.—Leeuwenhoek's observations remained practically isolated and without fruit for nearly a century. It was not until 1786 that the work of the Danish zoölogist, O. F. Müller, added anything of importance to the knowledge of bacteria. Müller recognized clearly the difficulties of studying such minute organisms. "The difficulties," he writes, in words that still appeal to the modern bacteriologist, "that beset the investigators of these microscopic animals are countless; the sure and definite determination [of species] requires so much time, so much acumen of eye and judgment, so much perseverance and practice, that there is hardly anything else so difficult." Despite the obstacles, however, Müller succeeded in discovering many structural details of which his predecessors had been ignorant. Indeed, he succeeded in depicting several kinds of bacteria so accurately that they can be identified today as belonging to one or another of the chief group-forms.

Another unequivocal advance was made by Ehrenberg (1795–1876). His principal work upon the "infusion animals," "infusoria," or "Infusionstierchen," as the animalcules found in infusions of hay, meat, and other organic substances were termed, was published in 1838, and brought together much more definite and detailed information concerning bacteria than had been previously secured. The chief merit of Ehrenberg's work lay in the system that it introduced into the study of micro-organisms. This investigator was able to establish a number of different groups among the organisms now called bacteria, and recognized clearly the fundamental differences between the larger forms, such as the screw-shaped or spirally-twisted organisms, and certain of the true protozoa with which they had heretofore been classed. Some of the names which Ehrenberg conferred upon his "infusion animals," such as bacterium and spirillum, are still current in bacteriologic nomenclature, although with changed signification.

In the two or three decades succeeding Ehrenberg's work considerable knowledge was amassed concerning the mode of development and physiology of bacteria, as well as their position in biologic classification, but the labors of Dujardin, Perty, Cohn, Nägeli, and others, although important, are quite overshadowed by the work of Pasteur.

Up to the period of Pasteur's investigations the rôle played by bacteria in various familiar natural processes, such as putrefaction, decay, and fermentation, had been, perhaps, vaguely suspected, but had not received conclusive demonstration. The memorable researches of Pasteur (1822–1895) upon spontaneous generation and fermentation imparted to the study of bacteria a broad biologic importance that it had not hitherto possessed. Bacteria and kindred micro-organisms were shown to be responsible for setting in motion and carrying out many every-day processes, the nature of which had not before been understood or which had been incorrectly assigned to "the oxygen of the air" or to other inorganic agencies. Putrefaction and decay were shown by Pasteur to be, not fields for the "spontaneous generation" of life, but manifestations of chemical disintegration due to the metabolic activities of micro-organisms engaged in satisfying their need of food. Fermentation was not due, as Liebig for a time maintained, to the presence of dead and dying yeast-cells which in the course of their own

molecular disintegration toppled over and dragged down certain complex organic molecules with which they were in contact, but, on the contrary, was caused by the effort of living and growing yeast-cells to satisfy their nutritional requirements.

It was almost entirely through the work of Pasteur that bacteria and their allies emerged from their relative obscurity as organisms chiefly of interest to the professional biologist and took a conspicuous position in natural science as a group of organisms whose activities and capabilities were full of a far-reaching significance for all mankind. If any one man can be looked upon as the founder of the science of bacteriology, that man is surely Louis Pasteur.

The profound importance of Pasteur's researches has been universally recognized. Lord Lister, whose own name is inseparably connected with the triumphs of antiseptic surgery, thus addressed Pasteur in 1892 at the latter's jubilee celebration: "Truly, there does not exist in the entire world any individual to whom the medical sciences owe more than they do to you. Your researches on fermentation have thrown a powerful beam, which has lightened the baleful darkness of surgery, and has transformed the treatment of wounds from a matter of uncertain and too often disastrous empiricism into a scientific art of sure beneficence. Thanks to you, surgery has undergone a complete revolution, which has deprived it of its terrors and has extended almost without limit its efficacious power."

Tyndall also has expressed in forcible words the sweeping change that was wrought in all conceptions of disease through the work of Pasteur. "We have been scourged by invisible thongs, attacked from impenetrable ambuscades, and it is only today that the light of science is being let in upon the murderous dominion of our foes."

If the researches of Pasteur mark the beginning of bacteriology, those of Robert Koch must be regarded as establishing bacteriology on the basis of an independent biologic science. In 1876 Koch brought forward convincing evidence that a specific bacterium (B. anthracis) was the cause of a specific disease in cattle (anthrax or splenic fever). The nature of the proof submitted in support of this view was so conclusive that it drew the attention of the scientific world, and incited many investigators to undertake similar researches along the line of the "germ theory." In 1882 Koch further conferred an inestimable benefit upon practical workers in this

field by his invention and application of solid culture-media, a technical device by which it became possible to isolate single species of bacteria and obtain them in pure culture. Prior to the introduction of solid media the isolation of a single species of microbe involved much difficulty and almost always a certain measure of uncertainty. So long as investigators were often not wholly secure as to whether they were dealing with a single species of bacteria or with a mixture of different kinds, the methods of work lacked uniformity and precision, and all general conclusions were hazardous. When, however, Koch showed how to obtain the descendants of a single living cell or cluster of cells free from extraneous matter and without admixture with other organisms, immediate advance became possible. It cannot be a mere coincidence that the great discoveries in bacteriology followed fast on the heels of this important technical improvement, and it is perhaps not too much to claim that the rise of bacteriology from a congeries of incomplete although important observations into the position of a modern biologic science should be dated from about this period (1882).

The Scope of Bacteriology.—As in other growing sciences, so in bacteriology a noticeable differentiation has occurred. The relation of bacteria to disease early took a conspicuous place among the subjects included within the scope of the new science, and it is highly probable that the side of bacteriology bearing upon the science of pathology and the art of medicine will always remain, what it is today, its most broadly important aspect. There is at present a tendency for the workers in this field to specialize either along strictly pathologic or along hygienic lines. In pathologic bacteriology consideration is given chiefly to the effects produced upon the animal body by the presence of bacteria and their toxins, to the distribution of the germs within the body, and to the reactions, defensive and otherwise, evoked by bacterial invasion. Hygienic or sanitary bacteriology deals more particularly with the channels by which bacteria leave the human body and pass into the outer world, with the mode and duration of life of disease germs in water, soil, and air, and with the avenues by which these disease germs are able again to approach and infect healthy individuals. No sharp line can be drawn between pathologic and sanitary bacteriology. A common meeting-ground of great importance is found in the researches upon immunity, where it is shown that the resist-

ance of the animal organism to infection depends both upon the nature of the tissues with which the germ comes in contact, and upon the hygienic surroundings of the organism with reference to food, temperature, moisture, and the like, as well as upon the inherited qualities of the various groups of body-cells. The interweaving of pathologic and sanitary bacteriology, of preventive and curative medicine, is illustrated with especial clearness in the chapter upon diphtheria (Ch. XV).

Although, from a practical point of view, the part played by bacteria in the causation of disease in man must be admitted to be of surpassing importance, it must not be forgotten that bacteria exert a marked influence upon the welfare of mankind in many other directions.

Bacteria not only disintegrate and destroy dead bodies, and attack and kill living organisms, but some forms are also constructive to a high degree, and translate important chemical elements, like nitrogen and carbon, from unavailable combinations into substances that can be utilized by higher forms of plant life.

It has been discovered, for example, that certain kinds of bacteria profoundly modify the composition of the soil and the character of crops, and are hence of importance to the agriculturist; that other kinds of bacteria impart the characteristic flavors or aromas to butter, cheese, and other dairy products; and that still others determine the success or failure of various industrial processes, such as the retting of flax, the tanning of hides, and, perhaps, the curing of tobacco. It is believed by many that the applications of bacteriology to various industries and manufactures and to agriculture are likely to become much more numerous in the near future.

Underlying all the applications of bacteriology are certain fundamental facts and principles concerning the structure, mode of development, and general physiologic requirements and capabilities of bacteria themselves. This subject-matter constitutes the ground-work of bacteriology, and is essential not only to a proper comprehension of the present practical applications of bacteriology, but also to the further development of the science.

Biologic Significance.—The fact should not be overlooked that bacteriology owes its present important place among the biologic sciences quite as much to its general scientific significance as to the success of its practical applications. It has been often pointed out

that bacteriology has produced a change in man's conceptions of the world around him so sweeping as almost to deserve the term revolutionary. Up to the middle of the nineteenth century the character of many of the most familiar of natural processes, such as decay, fermentation, and the like, was entirely misunderstood; contemporary spontaneous generation of at least the lower forms of life was the generally accepted belief of most scientific men; infectious diseases were not sharply differentiated from one another and the most fantastic hypotheses were advanced to explain their existence. Although the great mass of material phenomena elsewhere had been brought into apparent orderliness and system, here was a region in which the unscientific imagination rioted in mystery and extravagance. The penetration of this realm of obscurity by the discoveries of bacteriology gave the human race for the first time in its history a rational theory of disease, dispelled the myths of spontaneous generation, and set the process of decay and kindred phenomena in their true relation to the great cycle of living and non-living matter.

The new conception of the microscopic underworld which bacteriology brought into biologic science must be reckoned as a conspicuous landmark, and, in so far as it has changed the attitude of man toward the universe, should be regarded as one of the most important triumphs of natural science.

Some of the aspects of the historical development of bacteriology are admirably treated in two essays by Huxley: "Discourses, Biological and Geological," New York, 1894 (Yeast, p. 110: Biogenesis and Abiogenesis, p. 229). A fairly detailed history of bacteriology to 1887 has been written by Löffler, entitled "Vorlesungen über die geschichtliche Entwickelung der Lehre von den Bacterien," Leipzig, 1887.

CHAPTER II

METHODS OF STUDYING BACTERIA

The ubiquity of bacteria, their minute size, and their occasional high resistance to external influences gave them a prominent place in the controversy that raged in the middle of the nineteenth century over the question of spontaneous generation. It was then assumed that when organic fluids and infusions of various kinds were heated to the temperature of boiling water all life was killed. If, therefore, bacteria appeared in infusions which had first been heated and afterward supposedly been protected against the ingress of micro-organisms from the air, their advent was hailed as an instance of spontaneous generation. It was, perhaps, not unreasonable to suppose that if any kind of life developed from non-living matter, this might be expected to occur among organisms so relatively simple in structure as bacteria. The progress of investigation, however, showed that it was not altogether an easy matter either to free organic fluids and extracts from bacteria, or to prevent the entrance of germs from the air. In the endeavor to overcome these two difficulties a rudimentary bacteriologic technic was developed which laid the foundation for the latter discoveries of Pasteur and Koch. Thus the discovery that cotton plugs, while they allow the air to circulate freely, are an effectual barrier to the floating particles in the air (Schröder and v. Dusch, 1854) was the direct outcome of experiments on spontaneous generation. Modern bacteriologic technic still makes extensive use of the cotton plug in protecting culture-media, etc., against atmospheric contamination.

The need of freeing glassware, instruments, and nutrient media from all forms of life before beginning bacterial experimentation of any sort is the central point of bacteriologic method. The principles of sterilization may therefore first be considered.

Sterilization of Glassware and Instruments.—As a preliminary to sterilization glassware, especially when new, should be thoroughly cleansed by boiling in soapsuds, or by soaking for an hour or more in a chromic acid cleaning mixture:

Potassium dichromate	60 parts
or	
Sodium dichromate	52 "
Water	300 "
Concentrated sulphuric acid	460 "

The sulphuric acid is added slowly, with constant stirring.

After being thoroughly rinsed and dried, test-tubes and flasks are plugged with a good quality of ordinary non-absorbent cotton. The plug should completely fill the opening, but a tight plug is not only unnecessary but hinders easy manipulation. A simple method for test-tubes is to use enough cotton so that the tubes

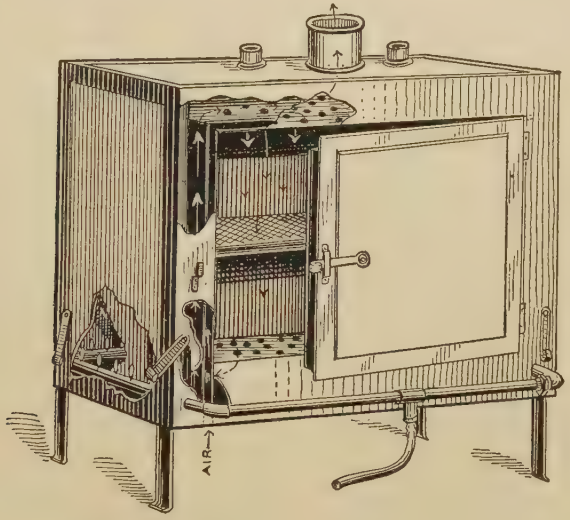


Fig. 2.—Lautenschläger hot-air sterilizer.

may be picked up by the plug. The plugged tubes and flasks are placed without crowding in a hot-air sterilizer. The best hot-air sterilizers, like the Lautenschläger pattern (Fig. 2), are fitted with thermometers and give a uniform temperature throughout. When the test-tubes and flasks are to contain media which is later autoclaved in them, it is only necessary to heat long enough to set the plugs. This is accomplished in fifteen to twenty minutes' exposure to a temperature of 170° to 190° C. For the destruction of all bacteria, even those in the resistant spore stage, forty-five minutes to one hour is sufficient. In many ovens and hot-air sterilizers

considerable temperature variation occurs in different parts, and this source of error must be guarded against. In the absence of exact temperature control a very slight browning of the cotton is sometimes taken as evidence that the necessary temperature has been reached. Too great charring must be avoided.

Pipets and Petri dishes, after thorough washing and drying, may be wrapped in paper or placed in metal cans before heating. Instruments may be sterilized directly in the flame or wrapped in manila paper and heated in the hot-air sterilizer at 170° C. for one hour. Scissors, forceps, knives, hypodermic needles, syringes, etc., should be boiled in water or in a 1 per cent. soda solution for three to five minutes before they are used, and after use boiled again thoroughly for disinfection. Platinum wires and loops for transferring bacteria from cultures are heated directly in a gas or alcohol flame until red hot, and then allowed to cool so that they will not injure the bacteria touched by them.

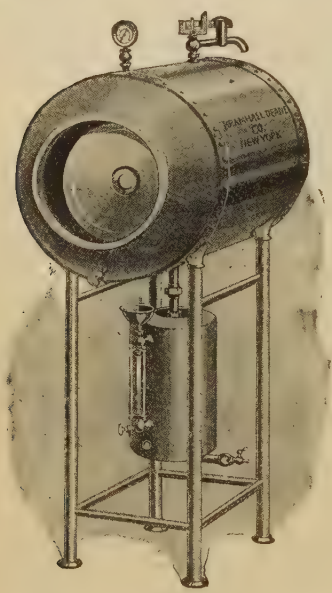


Fig. 3.—Autoclave. Horizontal form.

Rubber stoppers and tubing should be cleansed with soap and water and allowed to stand for one hour in 1 : 1000 mercuric chlorid solution, then washed with sterile water before using.

Autoclave Sterilization.—In order to effect immediate sterilization of culture-media, steam under pressure, and hence at a temperature higher than 100° C., is often used. The apparatus for this purpose, known as an autoclave, consists of a steam cylinder with a covered opening that can either be securely fastened or is held tight by the pressure within, a pressure-gage, safety valve, and sometimes a thermometer (Fig. 3). Steam is generated in a boiler underneath by means of a large gas burner or is supplied by direct connection with steam heating pipes. A temperature of 120° C. (15 pounds) for ten minutes is usually sufficient to sterilize completely all tubed

media; media in bulk should be heated for fifteen to twenty minutes. Care is necessary in the manipulation of the autoclave. (1) Baskets of tubes or flasks should not be piled on top of one another so that the stoppers become wet from the dripping. (2) All air should be allowed to escape before screwing down the stop-cock, as a mixture of steam and air does not reach the temperature indicated by the gage. (3) The pressure should be allowed to drop to zero before the stop-cock is opened, as a sudden removal of pressure may cause an explosive evolution of steam which will blow the stoppers and media out of the flasks and tubes. (4) In using an autoclave connected directly with a steam pipe care should be taken to see that the pressure is increased very gradually from zero to 15 pounds, otherwise the media in tubes and particularly that in flasks will not be heated to the proper temperature for the time given above. Gas-heated autoclaves work so slowly that there is no difficulty of this nature.

The autoclave is much used for sugar-free broth, agar, milk and gelatin, and for sterilizing test-tubes, apparatus, and discarded cultures. In many laboratories it is used for broths containing the simpler carbohydrates. A difference of opinion exists as to the desirability of such a procedure. The more complicated carbohydrates and most body fluids are best sterilized by the discontinuous method. In the preparation of Löffler's blood-serum it was the practice for many years to sterilize in an inspissator at 53° to 70° C., but this may be done in the autoclave with good results and less loss of time, provided sufficient care is taken to raise the medium slowly to the temperature of boiling water. In this way the air is driven from the medium before coagulation. Immersion of the tubes of blood-serum in warm water for half an hour before sterilizing, is advised.

Discontinuous Sterilization.—As just stated, certain kinds of media become to some degree unfit for bacteriologic work if subjected to the high temperature reached in the autoclave. The use of a lower temperature is hence desirable. Most bacteria are quickly killed by boiling. A serious drawback, however, to the use of simple boiling is the fact that very resistant bacterial spores are sometimes not killed even when boiled for several hours. The method of discontinuous sterilization is consequently adopted in some cases. Any simple apparatus may be used for this purpose,

such as a covered kitchen steamer over a pot of boiling water. A device in common use in the laboratory is the Arnold steam sterilizer (Fig. 4), which is constructed with a false bottom, so that a minimum volume of water is heated to produce steam quickly, while the main tank is constantly fed by the water of condensation, which is caught and collected by an outer jacket.

By the discontinuous method the medium is heated the first day for fifteen to twenty minutes after the steam has filled the sterilizer. The steaming process is repeated on one or two successive days, the medium being kept at 20° C. in the intervals. This method aims to kill by each steaming all those bacteria that are in the vegetative form, while the intervals of twenty-four hours are supposed to allow time for the resistant spores to develop into vegetative forms, which are destroyed at the next steaming. The method is not always successful. Smith[1] has pointed out that the spores of anaërobes may sometimes fail to germinate during the twenty-four-hour intervals in liquid media, such as the shallow layers of broth or milk where the liquid is well oxygenated, but remain dormant until favorable anaërobic conditions come about through the introduction and growth of film-producing organisms, when they germinate and a mixed culture results. Most flasks of media will remain sterile after four steamings if the intervals between are lengthened to forty-eight hours, but the autoclave may have to be brought into play in very obstinate cases.

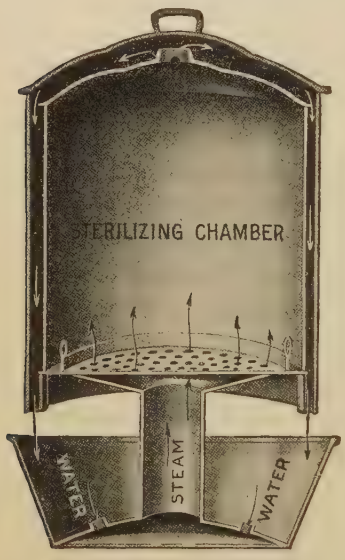


Fig. 4.—Arnold steam sterilizer (Fowler).

Preparation of Culture-media.—The food necessary for most micro-organisms is not of a highly complicated nature. Many species find suitable conditions for nourishment and multiplication if a small amount of simple nitrogen and carbon compounds and some salts and water are present. A slightly acid reaction to

[1] Smith: Jour. Exper. Med., 1898, 3, p. 647.

phenolphthalein (equivalent to a neutral or slightly alkaline reaction to litmus) and a temperature of about 20° C. afford excellent opportunity for the growth of micro-organisms in the presence of almost any ordinary food-stuff. A hydrogen ion concentration of 6.8 to 8.4 will permit the growth of most pathogenic species. It is largely by means of variations in the behavior of bacteria toward different carefully prepared nutrient substances that bacteriologists are able to differentiate the bacterial species. Many different culture-media have been devised which are in general laboratory use. The most common of these have for their basis an extract or decoction of meat to which a small amount of peptone is added. Koch found that by the addition of gelatin to this meat-peptone broth a solid, transparent medium could be obtained which greatly facilitated the study of the development of organisms. Gelatin, however, does not remain solid at 37° C., a temperature at which most pathogenic forms grow best, and, moreover, certain forms react upon the gelatin so as to produce liquefaction. To meet these conditions another gelatinous substance, of vegetable origin, agar,[1] which remains solid up to 100° C. and is not liquefied by bacteria, has been found to possess special advantages. Milk, potato, blood-serum, and nutrients to which blood is added complete the list of other media in common use.

Beef Broth.—500 grams (about 1 pound) of chopped lean beef[2] are placed in 1 liter of distilled water and kept in the ice-box overnight. It is then boiled for half an hour and filtered through paper or cheese-cloth. Ten grams of peptone[3] and 5 grams of salt are dissolved in the filtrate with as little heating as possible, the solution made up to a volume of 1 liter, and the reaction adjusted while hot by the addition of the required amount of normal sodium hydroxid. For the cultivation of many bacterial species meat extract may be used in place of meat infusion. In this case dissolve 3.5 grams of meat extract and 10 grams of peptone in 1 liter of water by gentle heating, make sure that the volume is correct, and then adjust the reaction.

The former practice of titrating media against standard alkali or acid is open to serious objection on chemical grounds. As pointed

[1] First used by Frau Hesse.

[2] Veal has been found preferable for some bacteria, particularly those species found in the upper respiratory tract.

[3] A number of brands of American made peptone are now obtainable.

out by Clark[1] and others the essential feature in the reaction of culture-media is the hydrogen ion concentration, and this is not accurately shown by the ordinary method of titration. In a word, the adjustment of different media to a "standard reaction" by the method of titration may give widely different hydrogen ion concentrations.[2]

The hydrogen ion concentration of a medium is expressed by use of the symbol P_H, as suggested by Sörensen. Numerically this is the logarithm of the reciprocal of the hydrogen ion concentration. Thus if C_H, the concentration of hydrogen ions, is 10^{-6} N (*i. e.*, $\frac{1}{1,000,000}$ N), and taking $P_H = \log \frac{1}{C_H}$ we have $P_H = \log \frac{1}{\frac{1}{1,000,000}} = \log 1,000,000 = 6.0$. For the neutral point $P_H = 7.0$; because of the reciprocal relation between P_H and hydrogen ion concentration, the number decreases with increasing acidity and increases with increasing alkalinity. The hydrogen ion concentration is most accurately determined by means of a potentiometer.[3] Furthermore, it has been shown that in different flasks or tubes of the same medium, sterilized at the same time, the final hydrogen ion concentration attained varies slightly. Therefore, other methods, less time consuming and requiring less elaborate apparatus, may be used for bacteriologic work. Sörensen[4] in the course of his studies on enzymes developed satisfactory colorimetric methods for determining hydrogen ions. These have been elaborated by Clark and Lubs[5] and applied to bacteriologic media. Others[6] have attempted to simplify the rather complicated standard solutions of these investigators. The following list of indicators, as suggested by Clark and Lubs, and by Cohen, includes the limits of the P_H values required for most media.

[1] Clark: Jour. Infect. Dis., 1915, 17, p. 109.

[2] Norton: Amer. Jour. Pub. Health, 1919, 9, p. 190.

[3] For a comprehensive discussion of methods for determining the P_H value see Clark: The Determination of Hydrogen Ions, Williams & Wilkins, Baltimore, 1922.

[4] Sörensen: Biochem. Zeitsch., 1909, 21, pp. 131, 201; 1909, 22, p. 352.

[5] Clark and Lubs: Jour. Bact., 1917, 2, pp. 1, 109, 196.

[6] Barnett and Chapman: Jour. Amer. Med. Assoc., 1918, 70, p. 506; Norton: Amer. Jour. Pub. Health, 1919, 9, p. 190; Medalia: Jour. Bact., 1920, 5, p. 441.

LIST OF INDICATORS

Common Name.	Concentration, Per Cent.	Range, Ph.
Thymol blue	0.04	1.2–2.8
Brom phenol blue	0.04	3.0–4.6
Brom cresol green	0.04	4.0–5.5
Methyl red	0.02	4.4–6.0
Propyl red	0.02	4.8–6.4
Brom cresol purple	0.04	5.2–6.8
Brom thymol blue	0.04	6.0–7.6
Phenol red	0.02	6.8–8.4
Cresol red	0.02	7.2–8.8
Thymol blue	0.04	8.0–9.6
Cresol phthalein	0.02	8.2–9.8

The method is as follows: Place 4 c.c. of neutral distilled water in a small (4-inch) test-tube, add 1 c.c. of the medium and 5 drops of indicator. Match the color obtained with one of a series of standards made in the same way from solutions of known hydrogen ion concentration or with color plates.[1] Various ingredients may be used in the preparation of these solutions. Those proposed by Clark and Lubs are the most satisfactory, but are difficult to prepare. Sörensen used $\frac{1}{15}$ molal solutions of potassium dihydrogen phosphate (KH_2PO_4) and disodium phosphate ($Na_2HPO_4.2H_2O$). The table below gives the amounts of each solution in a total volume of 20 c.c., corresponding to P_H values from 5.3 to 8.3.[2] Care must be taken to ensure the purity of the salts, particularly in the preparation of the sodium phosphate. This salt loses part of its water of crystallization on standing.

AMOUNTS OF SOLUTIONS ACCORDING TO P_H VALUE

Ph.	M/15 KH_2PO_4.	M/15 Na_2HPO_4.
5.3	19.5	0.5
5.5	19.2	0.8
5.8	18.4	1.6
6.0	17.5	2.5
6.2	16.1	3.9
6.4	14.5	5.5
6.6	12.3	7.7
6.8	10.0	10.0
7.0	7.8	12.2
7.2	5.7	14.3
7.4	4.0	16.0
7.6	2.6	17.4
7.8	1.6	18.4
8.0	1.0	19.0
8.3	0.5	19.5

[1] Clark: "The Determination of Hydrogen Ions," Baltimore, 1922.

[2] Sörensen: Biochem. Zeitschr., 1909, 22, p. 355.

The medium may be adjusted by trial or titrated in the following manner: Place 5 c.c. of the medium in 20 c.c. of neutral distilled water and add 10 drops of indicator (determined by the desired hydrogen ion concentration). From a buret add N/20 sodium hydroxid until the color matches a solution prepared in the same way from the P_H standards. The c.c. of N/20 alkali used, multiplied by 10, gives the quantity of normal sodium hydroxid necessary to adjust 1 liter of the medium. The reaction obtained should be checked by a test after adjustment.

After adjustment the broth is heated in a flask in the autoclave at 120° C. for ten minutes, allowed to cool again to bring down the precipitate, and its final reaction determined; then it is filtered, placed in flasks or tubes, and sterilized.

It is probable that the precise initial "reaction" of bacterial culture-media is not of so much importance as is sometimes assumed, at all events, so far as ability to multiply is concerned. The sensitiveness to hydrogen ion concentration supposed to be possessed by certain bacteria needs investigation by more accurate methods than those hitherto employed. It must not be forgotten that when growth has once started, bacteria themselves are the primary factors in determining the reaction of the medium. Little is known about the extent to which products of bacterial growth other than hydrogen ions interfere with continuance of all multiplication.

Dextrose-free Broth.—Meat, and also extract of meat, often contain a slight amount of muscle sugar or glucose. If sugar-free broth is required, a simple method of removing the muscle sugar is employed (Theobald Smith): 10 to 20 c.c. of a pure, young broth culture of B. coli is added to the infusion of meat, and incubated eighteen hours at 37° C. The broth is then boiled to kill the organisms and the preparation carried on as above. Control tests should always be made, since prolonged activity of B. coli may cause the development of indol or other products. Special broth media are prepared by adding 0.5 or 1 per cent. of dextrose, lactose, saccharose, or other carbohydrate to the sugar-free broth. Broth containing carbohydrate is preferably sterilized by the discontinuous method.

Gelatin.—Ordinary nutrient gelatin is prepared by adding from 10 to 12 per cent. of the best grade sheet gelatin to broth prepared as above. The medium should be heated over a water-bath or an

asbestos pad only long enough to dissolve the gelatin; it should be stirred constantly. It is then cooled to 60° C., the reaction adjusted, and an egg, dissolved in 30 c.c. of water, stirred slowly in. The medium is placed in a flask and cautiously heated in an autoclave to 10 pounds pressure for five minutes in order to coagulate the albumin. Filtration through a towel moistened with hot water should give a clear filtrate which can then be tubed and sterilized. Gelatin may be autoclaved for five minutes at 120° C. and still solidify if placed at once in the ice-box. In warm weather 12 per cent. of gelatin is necessary. Special care should be taken to prepare gelatin each time in a uniform way, since otherwise the bacterial growth will vary considerably due to the differences in the media.[1] The usual method of determining the gelatin-liquefying properties of bacteria is to study stab- or plate-cultures in this medium incubated at 20° C. for one to six weeks. The progressive liquefaction of the medium is noted at intervals. The form of growth, the extent or shape of the liquefied zone and the characteristics of the plate colony sometimes contribute important information concerning the metabolism of the organisms, and may aid materially in their identification. Rothberg[2] has suggested that organisms which do not grow rapidly or at all at 20° C. may be grown in gelatin at 25° to 37° C. (according to their temperature relations) for eighteen to forty-eight hours, and then a portion of the culture inoculated on the surface of gelatin in a test-tube and incubated for fifteen days at 20° C. The production of gelatinases (gelatin-liquefying enzymes) in the original culture will be evidenced by liquefaction in the subculture. A more rapid but somewhat less reliable method is to incubate gelatin cultures at 37° C. for eighteen to seventy-two hours (the gelatin becomes fluid at this temperature) and then plunge the cultures into ice-water. If no gelatinases have been produced and there has been no enzymatic liquefaction of the medium, the gelatin solidifies; if liquefying enzymes have been produced, the gelatin remains fluid or semi-solid.[3]

Agar.—To the standard beef broth add 1.5 per cent. of pure shredded agar. Complete solution of the agar requires a boiling

[1] Whipple: Tech. Quart., 1902, 15, p. 127.

[2] Rothberg: Abstracts of Bact., 1917, 2, p. 12. See also Rush and Palmer: Jour. of Bact., 1921, 6, p. 571.

[3] Levene and Carpenter (Jour. Bact., 1923, 8, p. 297) have suggested the use of formol amino acid titrations and measurements of viscosity changes as indices of liquefaction during the course of bacterial growth in gelatin.

temperature and some little time. This can be more easily accomplished if the agar is finely cut and allowed to soak over night in a small amount of water or if it is dissolved in a minimum quantity of boiling water before the hot broth is added. After solution it is necessary to restore the water evaporated and to adjust the reaction again. The filtration of agar through paper is unnecessary. A medium of sufficient clearness may be obtained if a wet towel is used for filtration and if the medium is kept near the boiling-point. Sterilize in the autoclave.

Dextrose and Lactose Agar.—To agar made with sugar-free broth 1 per cent. of dextrose or lactose may be added before sterilization in the Arnold steam-bath at 100° C. Other carbohydrates may be used in the same proportions. It has been customary to add sufficient litmus solution to the agar to give a distinct color, either before sterilization or, preferably, just before plating or inoculation. In the latter case the litmus solution must be sterile. In recent years it has been difficult to obtain a supply of satisfactory litmus, with the result that other indicators have come into use which have proved to be better than litmus. In the carbohydrate media either brom-cresol-purple[1] or Andrade's indicator[2] may be used. Since these indicators are stable they may be added before sterilization.

Milk.—Milk is a useful medium for determining the production by bacteria of acids or enzymes which precipitate or digest the casein, or act upon the lactose. Fresh milk, or in large cities "certified" milk, should be obtained, steamed for fifteen minutes in the Arnold sterilizer, and placed in the ice-box overnight to allow the cream to separate. The middle portion of the milk should then be siphoned off, avoiding both cream and sediment. The usefulness of milk as a diagnostic culture-medium is enhanced by the addition of an indicator such as brom-cresol-purple.[1] Milk may be sterilized for five minutes at 120° C. in the autoclave. Many prefer the Arnold steamer for three or four successive days. Skim milk powder may be used in place of fresh milk for routine laboratory work; 150 grams should be rubbed in a mortar with sufficient cold water to make a smooth paste, and the whole made up to 1 liter. It

[1] 5 c.c. of a 0.25 per cent. alcoholic solution per liter of medium.

[2] 0.5 per cent. acid fuchsin in distilled water, decolorized to a yellow by N/1 NaOH. 10 c.c. of this solution per liter of medium.

is necessary to sterilize the milk powder suspension for fifteen minutes at 10 pounds pressure.

Blood-agar.—This medium is especially useful for cultivating the organisms found in the respiratory tract, such as the pneumococci, streptococci, etc. Veal infusion broth-agar (P_H 7.4 to 7.6) is used as a base. The agar is melted, cooled to 50° to 55° C., and 5 per cent. of defibrinated blood added under aseptic conditions. Either horse, sheep, rabbit, or human blood may be used, depending on the particular organisms to be grown.

Blood-serum.—This medium is used mainly for cultivating the diphtheria bacillus. Usually beef blood is allowed to clot, and the straw-colored serum pipeted off either directly or after centrifuging. *Löffler's blood-serum mixture* consists of 3 parts of beef-serum mixed with 1 part of neutral broth containing 1 per cent. of dextrose. This may be sterilized in the autoclave provided that great care is taken to raise the temperature of the medium slowly in order to prevent the formation of bubbles. *Hiss serum-water* is made by adding 1 part of beef-serum to 3 of water and sterilizing in the Arnold for three successive days.

Potato.—Many non-pathogenic organisms grow readily and characteristically on potato. Of the several methods of preparing potato, the simplest is as follows: Cut a cylindric piece of potato 5 cm. long by means of an apple-corer. Halve this by a diagonal cut lengthwise. The pieces should be placed in cold running water for a few hours and then slipped into potato tubes so that they present a slant surface uppermost for inoculation. Special potato tubes are made with a constriction in the glass, which holds the potato about 1 inch from the bottom. It is well to fill the tube below with distilled water or broth to provide moisture. Sterilize in the autoclave. Heinemann[1] has devised a medium which has been found advantageous as a substitute for potato. The following medium also has been found to be useful, since yeasts and molds as well as the chromogenic bacteria develop readily on it: Cut 250 grams of potato into pieces about the size of a walnut, place in 500 c.c. of distilled water, and steam for one hour. Pour the liquid through a wet towel, add 1 per cent. dextrose and 1.5 per cent. agar, heat until dissolved, make up to 500 c.c. with distilled water, tube, and sterilize.

[1] Heinemann: Jour. Infect. Dis., 1907, 4, p. 283.

Synthetic Media.—Media whose exact chemical composition is known offer certain advantages for the careful study of bacteria. Environment may thus be reduced to its simplest terms, or varied definitely and at will. Certain bacteria will develop in water which has been redistilled in glass, and has a trace of $MgSO_4$ added. Others refuse to multiply in more complex solutions. One of the simplest synthetic or "non-protein" media is as follows:[1]

Redistilled water	1000 c.c.
Asparagin	2 gm.
$MgSO_4$	1 "
K_2HPO_4	1 "

Uschinsky's medium (Fränkel's modification):[2]

Water	1000 c.c.
Asparagin	4 gm.
Ammonium lactate	6 "
Na_2HPO_4	2 "
NaCl	5 "

For cultivation of organisms of the colon-aërogenes group Ayers and Rupp[3] have used the following medium containing inorganic nitrogen and lactose as the only available carbohydrate:

	Per Cent.
Sodium ammonium phosphate	0.4
Acid potassium phosphate	0.2
Lactose	1.0
Agar	1.5

At the time of plating there is added to every 100 c.c. of the above medium while hot 0.5 c.c. of a 1 per cent. alcoholic basic fuchsin solution and 0.5 c.c. of a freshly prepared 5 per cent. sodium sulfite solution. On this medium members of the colon-aërogenes group give medium-sized red colonies, usually surrounded by a deep red ring. Other organisms that develop give pink or uncolored colonies.

Special Media and Biochemical Tests.—A great variety of special media are used in connection with the study of different organisms, either because such media are particularly favorable to the growth of those organisms or because they reveal certain characteristic features and biochemical reactions. The addition of

[1] Jordan: Bot. Gaz., 1899, 27, p. 19; Jour. Expt. Med., 1899, 4, p. 627.

[2] Fränkel: Hyg. Rundsch. 1894, 4, p. 769. For other formulas see Erwin Smith, "Bacteria in Relation to Plant Diseases," Washington, 1905, p. 197.

[3] S. H. Ayers and P. Rupp: Jour. of Bact., 1918, 3, p. 433.

glycerol to nutrient agar, for example, favors the development of the tubercle bacillus. Certain organisms, of which the so-called influenza bacillus is the type, are favored by the presence of hemoglobin in the culture-medium, and are often designated on that account as the hemophilic bacilli.[1] A few media have a decisive differential value; the typhoid bacillus does not produce either gas or acid in lactose broth, whereas a closely allied bacillus, B. coli, found in the normal human intestine, actively ferments lactose. In general, the ability to ferment carbohydrates or substances like mannite and glycerol which are added to the ordinary sugar-free culture-media, constitutes one of the most important differential characters of bacteria.

The *reducing power* of bacteria may be measured by the loss of color of litmus (*e. g.*, in litmus milk) or of methylene-blue, or by the reduction of nitrates to nitrites. The reduction of nitrates may be determined in the following way: After four days' incubation at 37° C. in nitrate broth (0.1 per cent. peptone, 0.02 per cent. nitrite-free potassium nitrate) add to 3 c.c. of the culture in a clean test-tube 2 c.c. each of the following solutions: (1) Sulphanilic acid solution made by dissolving 8 grams of the purest sulphanilic acid in 1000 c.c. of 5N acetic acid (sp. gr. 1.041); (2) α-amidonaphthalene acetate solution prepared by dissolving 5.0 gram solid α-naphthylamine in 1000 c.c. of 5N acetic acid and filtering the solution through absorbent cotton. The development of a rose color indicates the presence of nitrites. An uninoculated tube of the medium should always be treated in the same way for a control. Nitrate solution may also be inoculated in the fermentation tube, where the evolution of gas indicates a still further reduction to free nitrogen gas.

The production of *indol* may be determined in Dunham's peptone solution (1 per cent. peptone and 0.5 per cent. NaCl in water). More satisfactory are the trypsinized media of Rivas[2] and Cannon.[3] After four days' incubation the presence of indol may be detected by cautiously adding 1 c.c. of Ehrlich's reagent so that it forms a layer on the surface of the medium. A red color (due to the formation of a compound soluble in amyl alcohol) indicates a positive

[1] See Davis: Jour. Infect. Dis., 1907, 4, p. 73.
[2] Rivas: Cent. f. Bakt., I, Orig., 1912, 63, p. 547
[3] Cannon: Jour. Bacteriology, 1916, 1, p. 535.

result. The test may be made quantitative. Ehrlich's reagent is prepared as follows:

Paradimethylamidobenzaldehyde	4 gm.
Alcohol, 96 per cent	380 c.c.
Concentrated C. P., HCl	80 "

The vanillin test[1] is a satisfactory substitute.

The Fermentation Tube.—As already pointed out, various forms of bacteria differ greatly in their ability to ferment carbo-

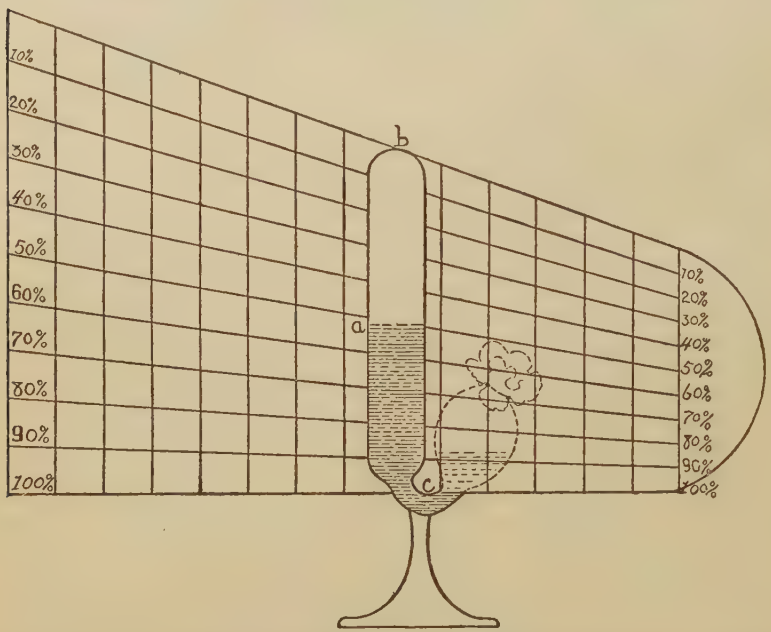


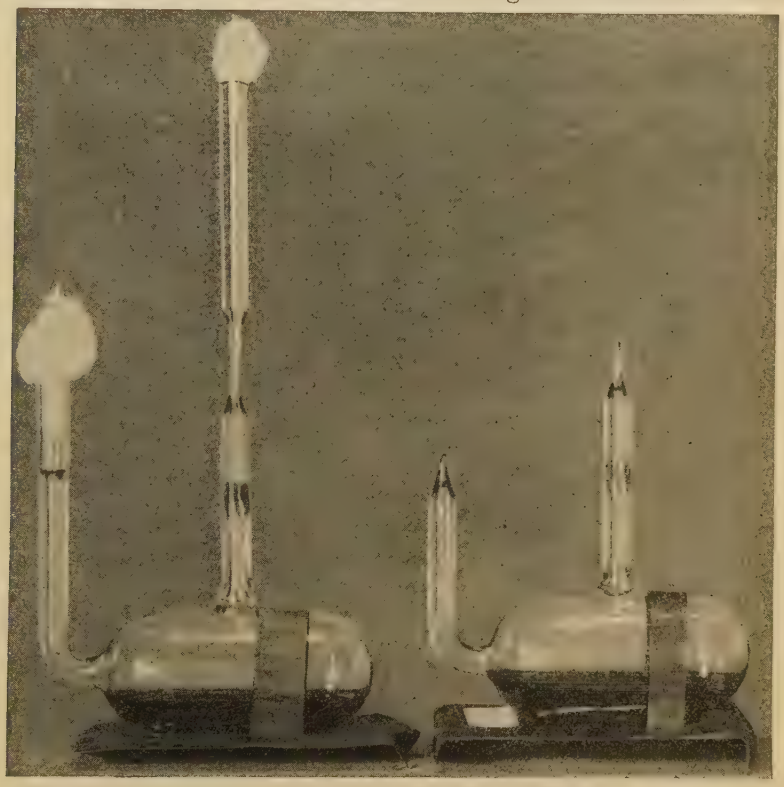
Fig. 5.—Frost's chart for measuring gas in the fermentation tests (Heinemann's "Laboratory Guide").

hydrate substances. The use of special tubes for studying fermentation and gas production was first recommended by Theobald Smith.[2] To determine the mere presence or absence of gas formation, the Dunham inverted vial is now generally used. Culture-tubes are one-third filled with broth from which the muscle-sugar has been removed and to which a definite amount, usually 0.5 to 1 per cent., of some carbohydrate has been added. An

[1] Nelson, V. E.: Jour. Biol. Chem., 1916, 24, p. 527.

[2] Smith, Theobald: "The Fermentation Tube," Wilder Quarter Century Book, Ithaca, 1893, p. 187.

inverted vial is then slipped into each tube. After sterilization it will be found that the air in the vial has been replaced by the broth. If the Smith tube (Fig. 5) is used it is completely filled with the carbohydrate broth. The growth of a gas-forming organism leads to the collection of gas in the closed arm, the displaced broth being forced out into the bulb. The amount of gas is measured in terms



Figs. 6 and 7.—Fermentation bulb (after Rogers, Clark, and Davis). The side arm serves for introduction of the medium and inoculation; it is then sealed off. The other tube is used for exhaustion of the air (by the Boltwood mercury pump) and is then sealed as shown.

of percentage of the length of the closed arm, most conveniently by Frost's gasometer card (Fig. 5). At the end of forty-eight hours the gas may be roughly analyzed in the following way: After the total quantity of gas is measured, the bulb is filled with a 2 per cent. NaOH solution and the mouth of the tube closed tightly with the thumb. The gas is tilted back and forth between the bulb and the closed arm several times and finally allowed to collect in the

closed arm. When the thumb is released the fluid rises in the arm, due to the fact that the sodium hydrate has absorbed the carbon dioxid; the residual gas, which is usually chiefly hydrogen, can then be measured. The ratio of hydrogen to carbon dioxid or the *gas formula* of an organism may be stated, for example, as follows:

$$H_2 : CO_2 : : 30 : 15 : : 2 : 1.$$

It is evident that results obtained in this manner can never be precise. The solubility of carbon dioxid leads to the retention in the medium of a varying percentage of this gas dependent on the acidity of the medium, the opportunities for loss into the atmosphere, and other factors. It has been shown, especially by Keyes,[1] that more uniform and comparable results can be obtained by using bulbs sealed after the exhaustion of the air. A modified form (Figs. 6 and 7) has been successfully used by Rogers[2] and his collaborators in a study of gas production in the colon group of bacteria.

Thermal Death-point.—The importance of knowing the condition and the time necessary for the destruction of bacteria has caused the introduction of certain exact methods for testing the death-point of various organisms subjected to the influence of heat and to the action of disinfectants. The heating test is best made by use of a small glass bulb devised by Sternberg (Fig. 8). To fill the tube, warm the bulb slightly to drive out the air and then insert the stem at once into the bacterial suspension, which is drawn by suction into the bulb as it cools. The neck is then sealed in the flame. An ordinary thin glass test-tube may be used if drawn out in the glass flame to form a narrow neck in the middle, through which a definite amount of culture may be run into the bottom of the tube. The neck is then drawn out in the flame and sealed off. Heating should be done with the bulbs completely immersed in a water-bath and held suspended by a wire away from the bottom frame. It is advisable to begin by exposing to a temperature of 50° C. for five minutes and then for ten minutes, repeating for every two degrees up to 70°. Spores require still higher temperatures for their destruction. After being cooled quickly the bulb contents are emptied into a Petri dish and melted agar added, to determine by the development of colonies the number of live organisms present. Eijkman[3] has called attention to an important error in such experi-

[1] Keyes: Jour. Med. Research, 1909, 21, p. 69.

[2] Rogers, Clark, and Davis: Jour. Infect. Dis., 1914, 14, p. 426.

[3] Eijkman: Centralbl. f. Bakt., ii, 1909, 22, p. 508.

ments. Many cells are so damaged by exposure to heat (and probably the same is true of other disinfecting agents) that they develop exceedingly slowly, although their vitality is not destroyed. In one experiment with Bacillus coli no colonies were visible on a gelatin plate within three days after incubation of the heated culture, but on the same plate after fifteen days 670,000 colonies could be enumerated. In order to discover whether all the organisms have been killed the contents of the bulb should be emptied, preferably into a fluid medium composed of equal parts of litmus milk and broth (Harris).

Fig. 8.—Sternberg's bulb for testing thermal death-point.

Methods for Testing and Standardizing Disinfectants.—The earlier attempts to determine the efficiency of disinfectants were carried out in a very simple fashion. Koch tested the action of disinfectants on anthrax spores by placing in the disinfectant solution silk threads which had been dipped in an emulsion of the spores and dried. The threads were then washed and laid upon the surface of agar. Hill[1] devised a simpler and more exact method of preparing test objects. Sterilized glass rods are dipped to a depth of 1 inch into forty-eight-hour-old broth cultures of the organism to be used in the test. The rods are then placed in test-tubes fitted with cotton plugs, carefully dried in the thermostat, and immersed in the disinfectant solution for accurately timed periods, varied as desired. Each rod, on removal, is gently but thoroughly washed with sterile physiologic salt solution, then placed in a tube of sterile broth, and incubated at 37° C.

There are so many factors concerned in such tests, however, that the results of different workers show great variation. The temperature at which the experiments are made, the number of bacteria, the nature of the culture-medium from which the bacteria are derived, and the solvent in which the disinfectant is employed all influence the results. Differences between bacterial species and between different strains of the same species are further complicating factors. Because of such modifying influences much of the earlier work on the efficiency of disinfectants contains inconsistencies and discrepancies.

[1] Hill: Rep. and Papers, Amer. Pub. Health Assoc., 1898, 24, p. 264.

The Rideal-Walker method first led to a more accurate standard of comparison.[1] The method consists essentially in determining what is known as the carbolic acid coefficient or, better, the phenol coefficient, since the carbolic acid of commerce may vary in the amount of phenol present. The phenol coefficient of a disinfectant is obtained by dividing the dilution of the disinfectant that kills an organism in a given time by the dilution of phenol that kills the same organism in the same time under exactly the same conditions.

A modification of the Rideal-Walker method was devised by Anderson and McClintic.[2] The phenol coefficient determined according to their method is known as the "Hygienic Laboratory phenol coefficient."

A still later method of standardization has been prepared by Reddish and is thought to combine the best features of the Rideal-Walker and Hygienic Laboratory tests while eliminating the objectionable features.[3]

Sterilization by Filtration.—It is often found desirable to sterilize water and other fluids, such as culture-media in which bacteria

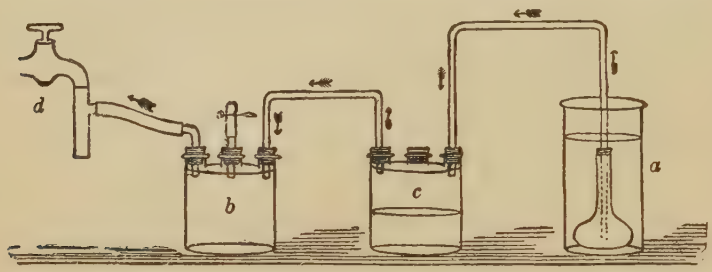


Fig. 9.—Apparatus for the rapid filtration of toxins, etc.: *a*, Filter flask; *b*, Woulff bottle to guard against regurgitation of water from the pump; *c*, reservoir for the filtrate; *d*, water vacuum pump (McFarland).

have been growing, without subjecting them to heat or to the action of disinfectants. The method of sterilization by filtration is especially useful in obtaining soluble bacterial products, such as toxins and enzymes, which might be injured by chemicals or heat. The bacterial products are separated from the bacteria by filtration of

[1] Rideal and Walker: Jour. Roy. San. Inst., 1903, 24, p. 424.

[2] Anderson and McClintic: Jour. Infect. Dis., 1911, 8, p. 1; Bull. 82, Hyg. Lab., U. S. Public Health Service, April, 1912.

[3] Reddish: Am. Jour. Pub. Health, 1927, 17, p. 320.

This is the report of the referee on Standardization of Disinfectants presented to the Committee on Standard Methods of the Am. Pub. Health Ass'n.

the culture through unglazed porcelain cylinders, which, together with the glass and rubber connections forming part of the filtration apparatus, have been previously sterilized by autoclaving. The porcelain cylinders in use for this purpose vary in form and in the size of their pores, one of the most commonly used being the "Chamberland B. pattern." The passage of fluids is usually very slow through the most compact cylinders, and may be hastened by a water suction-pump. An overflow bottle should be interposed between the filter and the tap to prevent any back-flow of water from entering the filter flask (Fig. 9). A very useful form of apparatus for filtering such fluids as blood-serum under pressure is shown in Fig. 10. The small Berkefeld filter with cylinders made of infusorial earth is most convenient for obtaining clear solutions, but the coarser grades of Berkefeld filters permit the passage of very small bacteria. Minute defects in the cylinders sometimes occur, and the filtrate should be tested for sterility by inoculation of a small amount into culture-media. Bacterial fluids after infiltration should be protected from light and kept in the ice-box.

Fig. 10.—Pressure filter (Heinemann).

Methods of Obtaining Pure Cultures.—When fluid culture-media are inoculated with such substances as soil or water, many kinds of organisms develop simultaneously side by side, and a heterogeneous mixture of bacteria results. Koch[1] was the first to devise a method of using solid media which permitted the separation of one kind of bacterium from another. If nutrient gelatin and agar are inoculated while fluid (for example, at 42° C.), and are then solidified and kept under favorable temperature conditions, many of the living bacteria that have been introduced are able to multiply. Since the bacteria cannot move about freely, but are fixed in the

[1] Koch: Mitth. a. d. kais. Gesundh., 1881, 1, p. 1.

stiffened medium, the progeny of each germ forms distinct masses or colonies. If the colonies are not closely crowded, a pure culture, that is to say, the descendants of a single germ, may be obtained by touching a colony with the tip of a sterile platinum needle (a process technically known as "fishing") and inoculating tubes of fresh culture-media. In order to secure a large surface upon which the colonies shall be spread out and made easily accessible, the gelatin or agar, after inoculation, is poured while still fluid into sterilized flat shallow dishes (Petri dishes) fitted with glass covers.

Technic of Making Plate Cultures.—Three tubes of agar (1, 2, and 3), melted at 100° C., are placed in a water-bath at 42° C., a temperature that is just above the solidifying point of agar and is not injurious to bacteria.[1] Tube 1 is inoculated with a platinum loopful of the material to be plated. The cotton plug is then replaced and the contents of the tube mixed by carefully tilting back and forth and rotating the tube on its long axis. From this tube two loopfuls of agar are transferred to tube 2, and after mixing, two more loopfuls carried from tube 2 to tube 3. The contents of the several tubes are then poured into Petri dishes. As soon as the cotton plug is removed, the mouth of each tube should be passed through the flame, inserted under the edge of the lifted Petri dish cover, and the agar quickly poured out. The covered Petri dish may then be tipped cautiously back and forth to distribute the agar evenly before it solidifies. Agar plates placed in the incubator after solidification should be inverted in order to avoid "spreading" of the growth through condensation of the moisture. If there are a great many bacteria in the original material, the plate from tube 3 will probably contain the organisms in small enough numbers to develop well-isolated colonies. On the other hand, if there are very few bacteria in the material inoculated, plate 1 will probably present more satisfactory conditions. Gelatin plates are made in the same manner as agar except that gelatin may be cooled as low as 25° C. without solidifying.

Dilution.—It is sometimes of advantage before plating to make accurate dilutions of highly polluted fluids, such as sewage, in order to get colonies few enough in number to be well isolated. If there

[1] It is often desirable to make first a suspension in salt solution, as, for example, in dealing with material like pus.

is reason to suppose that the number of bacteria is 200 per c.c., or more, 1 c.c. of the sample is mixed with 9 c.c. of sterile water. If a higher dilution is required, proceed in a similar manner.

(A)	To dilute	1 :	10	use	1 c.c. of sample	to	9	c.c. of	sterile	water.
(B)	"	1 :	100	"	"	"	" 99	"	"	"
(C)	"	1 :	1,000	"	"	(A)	" 99	"	"	"
(D)	"	1 :	10,000	"	"	(B)	" 99	"	"	"
(E)	"	1 :	100,000	"	"	(C)	" 99	"	"	"
(F)	"	1 :	1,000,000	"	"	(D)	" 99	"	"	"

Separation of Bacterial Species by Heat.—Spore-forming organisms are sometimes separated from other bacteria by heating mixed cultures containing spores to 80° C. for fifteen minutes. This procedure kills off any vegetative forms that may be present, but leaves the heat-resistant spores able to develop if placed under favorable conditions. A further separation of different varieties of spore-forming organisms must then be effected by plating or animal inoculation.

Separation by Animal Inoculation.—Certain pathogenic bacteria that often occur in the animal body mixed with other species, as is the case, for example, with B. tuberculosis or B. tetani, are sometimes obtained free from other bacteria by inoculating an animal with the material containing the mixture of organisms. After allowing time for the bacteria to develop, the animal is killed, and tubes of suitable media are inoculated from the characteristic lesions; in such cases the specific bacillus will often be found in pure culture in the tissues.

Method of Growing Anaërobes.—A number of devices have been used for the cultivation of certain organisms known as anaërobes (p. 82), which will not grow in the presence of free oxygen. Pasteur spread a layer of oil over the surface of media in order to shut off the air. Koch grew anaërobes on agar or gelatin plates under a piece of sterile mica. Liborius employed the method of inoculation deep into solid media, a simple method still in use. A "shake culture" in glucose agar may be made by previously boiling the agar for fifteen to twenty minutes to drive off the absorbed air, then cooling quickly to 42° C., and inoculating. The tubes of agar are then solidified at once in cold water and incubated; under these conditions anaërobic organisms often develop well in the depths of the agar.

Hydrogen Method.—Success is still more certain if the tubes are placed in a Novy anaërobic jar (Fig. 11). The air in the jar is replaced with hydrogen by allowing a full stream of washed hydrogen from a Kipp generator to pass through for ten to fifteen minutes before closing the stop-cock. The Novy jar is especially useful for making anaërobic plates.

Absorption of Oxygen.—Any vessel with a tight cover, such as a Novy jar, an ordinary chemical desiccator, or a Mason fruit-jar, may be used for Buchner's pyrogallic acid method. The principle of this method is the absorption of oxygen. Dry pyrogallic acid (10 grams per liter of air-space) is placed in the bottom of the jar, 150 c.c. of a 1 per cent. solution of NaOH poured on the pyrogallic acid, the cultures put in place, and the jar closed at once. This method may also be used directly in

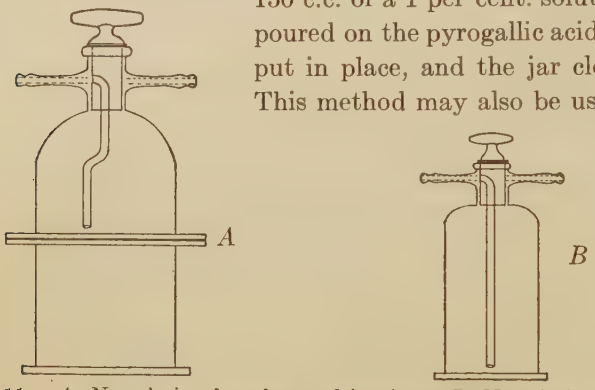


Fig. 11.—*A*, Novy's jar for plate cultivations; *B*, Novy's jar for tube cultivations (Eyre).

culture-tubes, a stopper of absorbent cotton being pushed down to seave an inch space at the top of the tube, pyrogallic acid and NaOH lolution placed in this space, saturating the cotton, and the tube closed at once with a tight-fitting rubber stopper.[1]

Vacuum Method.—An unpublished method first used by Gwyn in combination with the pyrogallic acid method has been found practicable by itself. The cultures are placed in a desiccator jar which has a cover with a single stop-cock. A piece of filter-paper saturated with alcohol is put into the jar, a lighted match applied, and the vaselined cover, with stop-cock closed, placed quickly in position. A good vacuum is formed, and if care be taken that the paper is well saturated with alcohol, no deleterious gaseous products of combustion are formed. It is necessary to open the cock before

[1] Wright, Jour. Bost. Soc. Med. Sci., 1900, 5, p. 114.

the cover can be removed. This is a simple and ready method if cultures are being made and examined often.

Wright[1] and Smith[2] have suggested good and simple methods of anaërobic culture in fluid media. Important points to observe in working with anaërobes are: (1) The culture-media should be freshly prepared. (2) The medium employed, whether gelatin, agar, or broth, should contain 1 per cent. glucose and should be freshly boiled and cooled before using. (3) The reaction of the medium should be nearly neutral to phenolphthalein.

At the present time the methods most in favor involve deep implantation in culture media, the use of fresh tissue media and, particularly for plate cultures, the anaërobic jar as described by Laidlaw,[3] McIntosh and Fildes,[4] and Richardson and Dozler.[5] In this jar the oxygen is removed by reacting with hydrogen in the presence of heated palladinized asbestos. The catalyzer is surrounded by a coil of nickel chrome wire through which an electric current is passed to provide the necessary heat.

A more detailed account of anaërobic methods will be found in the chapter on Pathogenic Anaërobes, as well as in a paper by Hall.[6]

Animal Inoculation.—Animal inoculations may be made for various purposes: (1) To obtain pure cultures of a pathogenic organism from contaminated material. (2) To determine the virulence of an organism which is under study or to observe the changes that it evokes in the animal body. The animals most used for laboratory purposes are guinea-pigs, rabbits, white mice, and white rats. (3) For continuing the life of an organism that does not grow except in the animal body (for example, the virus of hydrophobia and smallpox).

Guinea-pigs and rabbits are usually inoculated subcutaneously or intraperitoneally. Subcutaneous inoculation is generally made under the skin of the abdomen by means of a hypodermic needle. Pus or similar material may be suspended in sterile physiologic salt solution. The animal is conveniently held by an assistant, who turns its abdomen upward. The hair about the proposed site

[1] Wright: Jour. Bost. Soc. Med. Sci., 1900, 4, p. 119.
[2] Smith: Jour. Bost. Soc. Med. Sci., 1899, 3, p. 315.
[3] Laidlaw, P. P.: Brit. Med. Jour., 1915, 1, p. 497.
[4] McIntosh, J., and Fildes, P.: Lancet, 1916, 1, p. 768.
[5] Richardson and Dozler: Jour. Infect. Dis., 1922, 31, p. 617.
[6] Hall: Jour. Infect. Dis., 1920, 27, p. 576.

of inoculation should be clipped close. The site of inoculation is then rubbed with cotton soaked in 1 : 1000 HgCl or in 95 per cent. alcohol. Make the puncture behind and to one side of the umbilicus, and if a large quantity of fluid is to be injected, run the needle carefully forward its full length through the subcutaneous tissue. An egg-shaped swelling of the skin will form about the point of the needle as the syringe is emptied; when the needle is withdrawn, apply a drop of collodion to close the wound. "Pocket" inoculations are carried out by making a small incision in the skin, and separating the skin from the muscles by pushing in sterile scissors, which are then slightly expanded, closed again, and removed; a piece of tissue may then be inserted and the wound closed with collodion or with one or two sutures.

Intraperitoneal inoculation is made in essentially the same manner as subcutaneous, passing the needle first beneath the skin, then holding it at about a right angle to the peritoneal wall, and carefully thrusting it through, taking care not to penetrate the intestines. Rabbits may also be inoculated intravenously in the marginal vein of the ear. Rabbits are convenient animals to use in experiments where the blood is to be tested after inoculation, because of the readiness with which blood may be obtained from the large veins of the ears. Where 20 to 30 c.c. are needed it may be obtained from the heart.

Mice are usually inoculated subcutaneously on the back at the root of the tail. A small wire cylinder mouse-holder aids in manipulation.

Microscopic Methods.—*Examination of Living Bacteria.*—In order to determine form, motility, spore formation, and reaction with specific serum, it is often necessary to study bacteria alive. The hanging-drop method is commonly used for this purpose.

A special slide has been devised for the hanging drop which has a circular pit ground into the glass on one side. When bacteria are growing in fluid media, a drop may be transferred with the platinum loop to the center of a cover-slip which has been sterilized by flaming, and the cover-glass then inverted over the hollow chamber with the top depending freely downward. If the bacteria are removed from solid media, they should be suitably mixed with sterile physiologic salt solution and a drop of the suspension placed on the cover-slip. The hollow chamber is sealed by cedar

oil or vaselin smeared on the edge, so that the drop is not disturbed by air-currents and does not evaporate rapidly.

Extreme care must be taken in focusing upon the hanging drop, as unstained organisms are very difficult to see. The diaphragm of the microscope should be adjusted to a small aperture in order to take advantage of the lights and shadows caused by the difference in light transmission of the bacterial bodies. The edge of the hanging drop should first be found with a low-power lens and exactly centered, and then the high power turned in place and cautiously brought into focus.

Barber[1] has devised a method of making cultures from isolated single cells which has been used in the study of bacterial variability. The special technic is described in the articles cited.[2]

The so-called *hanging block* for studying the development of bacteria was invented by Hill.[3] It consists of a thin slice of nutrient agar (or of gelatin if a warm chamber is not necessary) which is seeded on its surface with a number of organisms and then inverted on a cover-glass and fastened by searing its edges with a hot needle. The cover-slip should be sealed over a moist chamber with paraffin. The organisms are thus held in one position on the solid medium, and the mode of cell division can be advantageously followed.

Young, vigorous cultures not more than twenty-four hours old should be used for studying cell division and also for determining motility in the hanging drop.

Examination of Stained Bacteria.—Film Preparation: Cover-slips and slides for making stained preparations should be thoroughly clean and sterile. If new, they should be washed in soapsuds or NaOH solution, boiled in potassium dichromate cleaning fluid, or placed in nitric acid (1 part concentrated HNO_3 to 1 part water) for one hour, rinsed well in distilled water, and stored in 95 per cent. alcohol or in alcohol and ammonia. They are then ready for use, and need only be dried with a clean soft linen cloth and freed from grease by passing them two or three times through the Bunsen flame. The cover-slip may be conveniently manipulated by means of special cover-slip forceps (Fig. 12).

[1] M. A. Barber: See Bull. Kansas Univ., 1907, 4, p. 3; Jour. Infect. Dis., 1908, 5, p. 380; 1909, 6, p. 634; Philippine Jour. Sci., 1913, 8, p. 539.

[2] R. Chambers Jour. Bact., 1923, 8, p. 1; H. W. Johnson: Jour. Bact., 1923, 8, p. 573.

[3] Hill: Jour. Med. Res., 1902, 7, p. 202.

Fresh young cultures twenty-four to forty-eight hours old grown on agar are usually the best for ordinary stained preparations. A loop of filtered water is placed on a cover-glass or slide, and a small amount of bacterial growth mixed with this and spread evenly over the surface. If the glass is not clean, the mixture will gather in droplets instead of spreading in an even film. This film is then allowed to dry in the air, and when well dried the preparation is passed three times slowly through the Bunsen flame, film side up, to fix the film. Fixing may be accomplished also by means of absolute alcohol or glacial acetic acid, which must be washed off before the next step. The fixed film is covered with the stain, allowed to stand fifteen to thirty seconds, washed thoroughly with clean water, and mounted in water for examination; if desired, it can be dried later and mounted permanently in balsam.

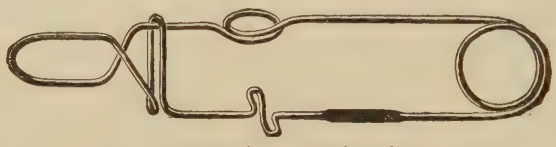


Fig. 12.—Novy's cover-glass forceps.

Stains.—The basic anilin dyes most commonly used for staining bacteria are, in order of merit, gentian-violet, methylene-blue, and fuchsin. Saturated solutions of these stains in 95 per cent. alcohol should be kept in stock, so that for immediate use it is only necessary to filter a little of the alcoholic solution into ten times its bulk of distilled water. Except in special cases (tubercle bacillus, spores, etc.), bacterial films stain very quickly with fuchsin or with gentian-violet. The action of methylene-blue may be hastened by gentle heating; some organisms, like the bacilli of glanders and of typhoid fever, take up a stain slowly and are best colored by the more intense stains. Methylene-blue is especially satisfactory for examining fluids from the body (blood, pus, etc.). The staining power of solutions may be increased by heating, by the use of substances which act as mordants, by prolonging the staining process, and by the addition of alkalis. Some of these special methods and stains are as follows:

Löffler's methylene-blue:[1]

Sat.sol. methylene-blue in alcohol	30 c.c.
Sol. KOH in distilled water (1 : 10,000)	100 "

[1] Löffler: Mitt. a. d. kais. Gesundh., 1884, 2, p. 421.

Anilin gentian-violet:

(*a*) Anilin oil water is made by adding 2 c.c. anilin to 98 c.c. distilled water; shake violently. Filter several times through filter-paper.

(*b*) Anilin oil water	75 parts
Sat. sol. gentian-violet in alcohol	25 "

Carbolic gentian-violet (Nicolle) is prepared as follows:

Gentian-violet (sat. alcoholic sol.)	10 c.c.
Carbolic acid	1 gram
Water	90 c.c.

Gram's Method:[1] Certain organisms, when stained and afterward treated with a solution of iodin and washed in 95 per cent. alcohol, give up the stain; others retain the color when subjected to this process. These latter organisms, examples of which are the anthrax bacillus and the pneumococcus, are said to be "gram-positive," or to "stain by Gram's method." Those losing the stain are "gram-negative." This method affords a means of measuring the relative permeability of micro-organisms to soluble substances. The gram-positive bacteria are those in which the iodin gentian-violet combination penetrates more or less deeply into the interior of the cell; the gram-negative varieties are only superficially affected.[2] Benians[3] interprets the phenomenon of gram staining as due to the possession of a definite cell-envelope which by the action of iodin is rendered impermeable to alcohol. This observer's experiments show that so long as the gram-stained cell is intact the alcohol is unable to remove the stain, but that as soon as the cell is crushed and injured the stain is, in great part, dissolved out. The amorphous débris obtained from broken-up gram-positive bacteria does not retain Gram's stain.

The procedure for Gram's method is as follows:

1. Stain in anilin gentian-violet for one and one-half minutes.
2. Wash in water.
3. Treat with Gram's solution (iodin, 1 part; potassium iodid, 2 parts; water, 300 parts) until purplish black, one and one-half minutes.

[1] Gram: Fortschr. d. Med., 1884, 2, p. 185.
[2] Brudny: Centralbl. f. Bakt., II, 1908, 21, p. 62.
[3] Benians: Jour. Pathol. and Bacteriol., 1912, 17, p. 199.

4. Decolorize with 95 per cent. alcohol for not more than two minutes, wash, dry, and mount.

A contrast stain with dilute fuchsin or 0.5 per cent. solution of safranin or Bismarck brown may be made if desired (for example, in examining gonorrheal pus); twenty-four-hour agar cultures should be used. For sections, prolong the process. Modifications: Weigert,[1] Nicolle,[2] Atkins.[3]

Sterling's Gentian-violet.—Five grams of gentian-violet are ground in a mortar with 10 c.c. of 95 per cent. alcohol. After practical solution, 2 c.c. of anilin oil are added and then 88 c.c. of distilled water. The grinding is continued a short time and after the mixture is permitted to rest a day or two, it is filtered through paper. It has the merit of staining quickly and intensely and of keeping many months.

The film, air-dried and passed through the flame, or preferably fixed in methyl alcohol, is stained as follows: The gentian-violet is applied for five seconds and washed off. The Gram's solution is applied for half a minute, and after washing or blotting away the excess, the preparation is decolorized and counterstained as above.

Pappenheim's Stain: A good general stain for pus preparations:

Sat. aqueous sol. of methyl-green	3–4 parts
Sat. aqueous sol. of pyronin	1–1½ "

Apply cold for thirty seconds.

Bacteria are stained bright red and the nuclei of cells blue or purple.

Stain for Tubercle Bacilli and Other "Acid-fast" Bacilli: Tubercle bacilli require a powerful stain containing a mordant, and with this aid stain only with difficulty; when once stained, however, they resist decolorizing with equal tenacity. The following method is most commonly used:

Ziehl-Neelsen Carbol-fuchsin Stain:[4]

Basic fuchsin	1 part
Absolute alcohol	10 parts
5 per cent. sol. carbolic acid	100 "

[1] Weigert: Fortschr. d. Med., 1887, 5, p. 228.
[2] Nicolle: Ann. de l'Inst. Past., 1895, 9, p. 666.
[3] Atkins: Jour. Bact., 1920, 5, p. 321.
[4] Ziehl: Deut. med. Wochenschr., 1882, 8, p. 451.

1. Flood the cover-glass with carbol-fuchsin and heat gently over the flame until the film seems deeply stained.

2. Wash and decolorize with a 2 per cent. solution of hydrochloric acid in 80 to 95 per cent. alcohol. It is well to decolorize until the thinner portions of the film show no red color.

3. Wash in water.

4. For a contrast stain use methylene-blue.

5. Wash and examine.

Möller's Spore Stain:[1] Like the tubercle bacillus, bacterial spores are resistant to staining and to decolorizing. The method of treating them is as follows:

1. Prepare films from a twenty-four-hour agar culture.

2. Place in chloroform for two minutes.

3. After drying in the air, cover with a 5 per cent. solution of chromic acid for two minutes.

4. Wash thoroughly in water.

5. Cover with carbol-fuchsin, and heat for five minutes over the water-bath at 100°, or over a small flame, simmering gently.

6. Decolorize with 1 per cent. sulfuric acid for twenty-five to thirty seconds.

7. Wash thoroughly in water.

8. Mount in water and examine under the microscope to see if the spores are cherry-red and the protoplasm colorless or faintly pink.

9. Counterstain with methylene-blue for ten to fifteen seconds without heat.

10. Wash, examine in water, and mount in balsam.

The body of the cell should appear blue; the spore, red.

Capsule Stain: Welch's method[2] is used for staining encapsulated organisms.

1. Cover the film with glacial acetic acid.

2. Draw off acetic acid and treat the film several times with anilin gentian-violet.

3. Wash in 0.85 per cent. NaCl solution and examine in the same solution. Avoid the use of water at any stage. The capsule appears as a pale violet halo around the deeply stained bacterium.

[1] Möller: Centralbl. f. Bakt., 1891, 10, p. 273.

[2] Welch: Bull. Johns Hopkins Hosp., 1892, 3, p. 128.

Hiss's Capsule Stain: Preparations are best made by direct films from pneumococcic exudates. Dry in air and fix by heat. Stain for a few seconds with saturated alcoholic solution of fuchsin or gentian-violet (5 c.c.), in distilled water (95 c.c.). Flood the slide with the dye and hold the preparation for a second over a free flame until it steams. Wash off the dye with 20 per cent. aqueous copper sulphate solution. Blot (do not wash in water).

By this method the capsule appears as a faint blue halo around a dark purple cell body. Better results may frequently be obtained by omitting heat fixation and by washing off the dye with the copper sulphate solution as soon as it begins to steam. Water should not be applied at any stage of the procedure.

Flagella Stain: For staining the flagella of bacteria, young agar cultures of twelve to eighteen hours should be used. Care must be taken to treat the organisms gently, since under some conditions the flagella are easily broken off. A tube containing 5 c.c. of sterile water is carefully inoculated with enough of the culture to produce a faint turbidity. The tube is then placed in the thermostat for an hour to allow clumps to sediment and to permit slight development. Two or three loopfuls of this suspension are then placed upon a cover-slip *without spreading*, dried at 37°, and fixed.

Löffler's stain,[1] in which a mordant is used, gives good results.

1. Cover the film with the mordant:

Tannic acid (25 per cent. aqueous solution)	10 parts
Ferrous sulfate (saturated aqueous solution)	5 "
Fuchsin (saturated alcoholic solution)	1 part

2. Heat over a water-bath for five minutes; keep the cover-slip flooded with the mordant.

3. Wash thoroughly in water and dry with filter-paper.

4. Cover the film with anilin gentian-violet or carbol-fuchsin and heat as before.

5. After washing thoroughly in water the film is ready to be mounted and examined.

In *Van Ermengem's method*[2] three solutions are necessary, as follows:

Solution A—

Osmic acid, 2 per cent. sol	1 part
Tannin, 10 to 25 per cent. sol	2 parts

[1] Löffler: Centralbl. f. Bakt., 1889, 6, p. 209; 1890, 7, p. 625.

[2] Van Ermengem: Centralbl. f. Bakt., 1894, 15, p. 969.

Place the films in this for one hour at room temperature or heat over water-bath at 100° for five minutes. Wash with water, then with absolute alcohol, then with water, and treat with

Solution B—

0.5 per cent. sol. of $AgNO_3$ in distilled water.

Allow films to be in this a few seconds; then, without washing, transfer to

Solution C—

Gallic acid	5 gm.
Tannin	3 "
Fused potassium acetate	10 "
Distilled water	350 c.c.

Keep in this for a few seconds. Then treat again with solution B till the film begins to turn black. Wash and examine.

Romanowsky Stain: This stain, depending upon a combination of eosin with altered methylene-blue, is extensively used in studying protozoan parasites.

Solution A[1]—

Methylene-blue (medicinally pure)	2 gm.
Sodium bicarbonate	9 "
Distilled water	25–30 c.c.

Mix the dry ingredients and gradually add the water. Cover and steam in the Arnold sterilizer one and one-quarter hours. Wash the residue with water, to remove the sodium bicarbonate, add 10 c.c. of 4 per cent. solution of NaOH, and shake. Extract with chloroform and evaporate over a water-bath. Finally, place the dry mass in a bottle and add gradually about 150 c.c. methyl alcohol. This constitutes the stock solution of crude methylene-violet and azure.

Solution B—

Saturated solution of Grübler's watery yellow eosin in methyl alcohol.

To make the dye:

Solution A	66 c.c.
Methyl alcohol	33 "
Solution B	1–1.5 "
Methylene-blue	0.05–0.15 gm.

[1] After Harris: Bull. Johns Hopkins Hosp., 1907, 18, p. 281. For fuller details see MacNeal: Jour. Infect. Dis., 1906, 3, p. 412.

Place the dye on the cover-slip and allow it to stand one minute. Add, drop by drop, a quantity of water equal to the bulk of the dye, and allow this to stand five minutes. Wash one-half minute in running water.

Epstein's stain is very useful in showing the granules in diphtheria bacilli:

(*a*) Prepare a film in the usual manner.

(*b*) Stain with Löffler's methylene-blue for twenty seconds.

(*c*) Wash in water.

(*d*) Warm over a flame with Gram's iodin solution.

(*e*) Wash in water.

(*f*) Dry and mount.

Neisser's Granule Stain for Diphtheria Bacilli:

Solution No. 1

Methylene-blue	0.1 gm.
Alcohol	2 c.c.
Glacial acetic acid	5 "
Distilled water	95 "

Dissolve the methylene-blue in the alcohol and add it to the acetic acid water mixture. Filter.

Solution No. 2

Bismarck brown	0.2 gm
Water (boiling)	100 c.c.

Dissolve the stain in the boiling water and filter.

To Stain: Fix the preparation. Pour on the dilute acetic acid methylene-blue solution and allow to act from thirty to sixty seconds. Wash. Then pour on the Bismarck brown solution and after thirty seconds wash off with water. Dry and mount. The bodies of the bacilli are brown with dark blue dots at either end.

Ponder's Stain for Diphtheria Bacilli:

Toluidin blue (Grübler)	0.02 gm.
Glacial acetic acid	1 c.c.
Absolute alcohol	2 "
Distilled water to	100 "

This stain may be used in place of Solution No. 1 in Neisser's method.

Wright's Stain:

Methylene-blue	1 gm.
Sodium carbonate	0.5 "
Eosin, yellow, water soluble	0.1 per cent. solution

The sodium carbonate is dissolved in 100 c.c. of distilled water, the methylene-blue added, and the solution placed in a steam sterilizer for one hour. When cold, 500 c.c. of the eosin solution is added and stirred constantly until the solution becomes deep purple and a finely divided precipitate has formed. This is collected on a filter paper, dried, and dissolved in methyl alcohol to form a saturated solution. After filtering, 40 c.c. of the solution are diluted to 50 c.c. with methyl alcohol. In using, cover the prepared film with the stain for one minute, then add water slowly until a green iridescence appears. Allow the stain to remain for two minutes, then wash and examine.

Jenner's Stain, Leishman's Modification.—This stain contains the same ingredients as Wright's stain, but is prepared in a different manner. The methylene-blue is dissolved in 100 c.c. of distilled water, the sodium carbonate added, the solution heated at 65° for twelve hours, and then allowed to stand ten days at room temperature. Equal amounts of this solution and the eosin solution are mixed and allowed to stand for six to ten hours with frequent stirring.

The precipitate is collected on a filter, dried, and the staining solution made by dissolving 0.15 gm. in 100 c.c. of methyl alcohol.

Giemsa Stain:

Azur II—eosin	0.3	gm.
Azur II	0.08	"
Glycerol	25	c.c.
Methyl alcohol	25	"

Heat both the alcohol and glycerol to 60° C. Dissolve the dyes in the alcohol and add the glycerol slowly. Allow to stand over night and filter. In staining use 1 : 1000 potassium hydroxid solution in place of water, and dilute the stain as prepared, with ten times its volume of water. The best preparations are made by allowing the stain to remain on the film for one to three hours.

The above three stains are useful in connection with the examination of blood smears either for making blood counts or for detecting parasites.

Selective Bactericidal Action of Gentian-violet.—Churchman[1] has used the method of divided plates for determining the difference in the behavior of bacteria to gentian-violet. An ordinary Petri dish

[1] Churchman: Jour. Exper. Med., 1912, 16, p. 221.

is divided into two compartments by a strip of metal, and, after sterilization, plain nutrient agar is poured into one, gentian-violet agar (1 : 100,000) into the other. On streaking the surface of the hardened agar (with a fluid culture or emulsion) striking differences in behavior are observed, which are of significance in differential diagnosis. Certain bacteria (*e. g.*, Bacillus anthracis, B. diphtheriæ) are inhibited by the dye and refuse to cross the border-line, while others (*e. g.*, B. typhosus, V. choleræ) grow freely in the gentian-violet medium. In general, the gram-positive organisms are inhibited by the gentian-violet and vice versa, but there are some exceptions to this rule. Behavior towards gentian-violet is much more definite and constant than the gram-staining reaction. The behavior of bacteria towards acid fuchsin is in many respects the exact opposite of their behavior toward the basic dyes. Besides its diagnostic value, the bacteriostatic method has important practical applications in detecting mixtures of species, air contamination, and the like. The selective action of the anilin dyes has been utilized therapeutically.[1]

Study of Pathologic Material.—The examination of material such as pus, blood, or discharges from diseased tissues or organs, may be carried out as follows: (1) Gelatin and agar tubes should be inoculated and plates poured at once. If there is reason to suspect that bacteria may be present that cannot develop on the ordinary media, blood-serum or blood-agar should also be inoculated. (2) Several cover-glass preparations should be made at the same time, one to be stained with methylene-blue, one with Ziehl-Neelsen carbol-fuchsin, and one by Gram's method. (3) A guinea-pig or rabbit should be inoculated with a small amount of the material.

The colonies that appear on the plates should be carefully examined, and pure cultures on agar or blood-serum made from any that present characteristic appearances, or that differ materially from one another. If the inoculated animal succumbs, cultures should be made from the site of inoculation, from the heart, spleen, liver, and peritoneal cavity. When no apparent effect follows inoculation, the animal should be killed after five or six weeks, the organs carefully scrutinized for possible tuberculous or other lesions, and cultures made as above.

[1] Churchman: Jour. Amer. Med. Assoc., 1922, 79, p. 1657.

Study of Pure Cultures.—In order to determine the natural relationship or systematic position of a bacterium it is necessary to observe in pure culture the morphology, staining reactions, mode of growth on various media, the biochemical reactions produced by its development, and in some cases the effect upon animals.

1. *Morphologic Appearance.*—Young twenty-four-hour-old agar cultures should be examined in a hanging drop for motility, form, and size. The hanging block may be used for further observations on size, cell division, grouping, and spore formation. Preparations of both young and old cultures should be stained first by the ordinary dyes, then by special methods, for the purpose of determining the gram stain reaction, the number and arrangement of flagella and spores, and the absence or presence of capsules.

2. *Cultural Characteristics.*—Cultures freshly isolated from water, soil, or air, or those which have been kept for long periods upon artificial media, are sometimes put through a process of "rejuvenation" before study in order that they may regain lost qualities. This process consists in successive transplantations upon a series of media, *i. e.*, from agar to broth for three days' incubation at 20° C., then to a gelatin plate for the same interval, and finally back to agar, from which the usual media may be inoculated.[1] Observations of cultural features should cover a period of at least ten days. The colonies on gelatin, and less often on agar plates, sometimes show important differences in form, rapidity of development, elevation, character of periphery, and internal structure as seen under a low-power lens. Agar and potato streak cultures should be examined with reference to form, amount and consistency of growth, elevation, character of surface, edge, and pigmentation or discoloration of the medium. In growth upon blood-serum the same features are to be noted, and also the occurrence of liquefaction or digestion. Gelatin stab-cultures often give a characteristic development along the line of puncture; observations should also be made on the character of the surface growth and the progress of liquefaction if present. The production of a surface pellicle, of turbidity and sediment in broth, and the occurrence of coagulation, digestion, and nature of reaction in milk are the features of growth in liquid media that differ with different species. An important biochemical property is the power of fermentation of dextrose, lac-

[1] Fuller and Johnson: Jour. Exper. Med., 1899, 4, p. 609.

tose, and saccharose, and in case of gas production, the amount of gas produced, and the ratio of H to CO_2. Growth in the closed arm of the fermentation tube gives an indication of facultative or obligatory anaërobiosis. It is sometimes considered useful to determine also whether a micro-organism possesses the ability to reduce nitrates in nitrate broth, and to form indol in peptone solution. The virulence of the organism itself and the toxicity of its products of growth (in the form of culture-filtrates) for ordinary laboratory animals complete the usual list of data necessary for classification.

It is of the utmost importance that descriptions be comparative; therefore, in order to prevent confusion of methods and of terms, a committee[1] on Bacteriological Technic, of the Society of American Bacteriologists, has suggested certain standard methods and descriptive terms, together with a numerical system of recording salient characters of an organism. The chart devised by this committee is herewith appended.

[1] Committee Reports, Soc. Amer. Bact.; Jour. Bact., 1918, 3, p. 115; 1919, 4, p. 107; 1920, 5, p. 127.

Endorsed by SOCIETY OF AMERICAN BACTERIOLOGISTS
at the annual meeting Dec. 30, 1920

DESCRIPTI

Name of organism.....Source.....Date of isolation...

INVIGORATION OF CULTURE: Date.....Medium used.....Temperature

MORPHOLOGY

NOTE—Underscore required terms.

SKETCHES

VEGETATIVE CELLS, Medium used.....
temp.....age.....days.
Form, *spheres, short rods, long rods, filaments, commas, short spirals, long spirals, curved.*
Arrangement, *single, pairs, chains, fours, clusters, cubical packets.*
Limits of Size.....Size of Majority.....
Ends, *rounded, truncate, concave..*

CAPSULES, present on.....
How stained.....

SPORANGIA, *present, absent.* Medium used.....
temp.....age.....days.
Form, *elliptical, short rods, spindled, clavate, drumsticks.*
Limits of Size.....Size of Majority.....

ENDOSPORES, *present, absent.*
Location of Endospores, *central, polar.*
Form, *spherical, elliptical, elongated.*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin.*
Sporangium wall, *adherent, not adherent.*

MOTILTY
In broth..... On agar.....
FLAGELLA, No. .....Attachment, *polar, bipolar, peritrichiate.* How stained.....

IRREGULAR FORMS,
Present on.....in....days at.....°C.
Form *spindled, cuneate, filamentous, branched,* or .....

STAINING REACTIONS.
Gram.....Acid fast.....
Special stains.....
.....
.....
.....

CULTURAL CHARACTERISTICS

	Underscore required terms.	SKETCHES	Underscore required t	
Agar Stroke Incubation Temperature .....°C Age .....d	Growth, *scanty, moderate, abundant, none.* Form of growth, *filiform, echinulate, beaded, spreading, arborescent, rhizoid.* Elevation of growth, *flat, effuse, raised, convex.* Lustre, *glistening, dull.* Topography, *smooth, contoured, rugose.* Optical Characters, *opaque, translucent, opalescent, iridescent.* Chromogenesis.....Photogenic. Fluorescent. Odor, *absent, decided, resembling*..... Consistency, *butyrous, viscid, membranous, brittle.* Medium, *grayed, browned, reddened, blued, greened.*		Nutrient Broth Temperature .....°C Age .....d	Surface growth, *ring membranous, none.* Clouding, *slight, mo sient, persistent, n* Odor, *absent, decided* ..... Sediment, *compact, flaky, viscid on a scant, none.*
Gelatin Stab Temperature .....°C Age .....d	Growth, *uniform, best at top, best at bottom.* Line of puncture, *filiform, beaded, papillate, villous, arborescent.* Liquefaction, *none, crateriform, napiform, infundibuliform, saccate, stratiform;* begins in.....d complete in.....d. Depth of liquefaction in tube of 10 mm. diameter evenly inoculated at 20° C. for 30 days.....mm. Medium, *fluorescent, browned.*		..... Medium (liquid) Temperature .....°C Age .....d	
..... Medium (solid) Temperature .....°C Age .....d			..... Medium Temperature .....°C Age .....d	

E CHART

Prepared by H. J. Conn, K. N. Atkins, I. J. Kligler, J. F. Norton, G. E. Harmon. } *Committee on bacteriological technic*

.....Studied by.....Culture No.....

.....Number of transfers.....Length of each incubation....days. Series No....

Index No.*.....

BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below:

PRIMARY CHARACTERISTICS

Microscopic Features

Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinæ; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous

Spores: 1, central; 2, polar; 3, absent

Flagella: 1, peritrichic; 2, polar; 3, absent

Gram stain: 1, positive; 2, negative

Miscellaneous Biochemical Reactions

Pathogenicity, etc.: 1, for man; 2, for animals; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic

Relation to oxygen: 1, strict aerobe; 2, facultative anaerobe; 3, strict anaerobe

Gelatin liquefaction: 1, positive; 2, negative

In nitrate media: 1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas

Chromogenesis: 1, flourescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none

Carbohydrate Reactions

Diastatic action: 1, positive; 2, negative

From dextrose: 1, acid and gas; 2, acid without gas; 3, no acid

From lactose: 1, acid and gas; 2, acid without gas; 3, no acid

From sucrose: 1, acid and gas; 2, acid without gas; 3, no acid

SECONDARY CHARACTERISTICS

Vegetative Cells

Diameter: 1, under 0.5μ; 2, between 0.5μ and 1μ; 3, over 1μ

Length: 1, less than 2 diameters; 2, more than 2 diameters

Chains (4 or more cells): 1, present; 2, absent

Capsules: 1, present; 2, absent

Spores

Shape: 1, round; 2, oval to cylindrical

Diameter: 1, less than diameter of rod; 2, greater than diameter of rod

Cultural Features

Agar Stroke

Abundance: 1, abundant; 2, moderate; 3, slight; 4, absent

Lustre: 1, glistening; 2, dull

Surface: 1, smooth; 2, contoured; 3, rugose

Agar colonies: 1, punctiform; 2, round (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled

Gelatin colonies: 1, punctiform; 2, round (over 1 mm.); 3, irregular; 4, filamentous

Milk

Acid: 1, sufficient for curdling; 2, insufficient for curdling; 3, no acid

Rennet curd: 1, present; 2, absent

Peptonization: 1, present; 2, absent

*Recording the "Index Number" here is optional; but its use will be found convenient if the charts are to be filed according to the salient characteristics of the organisms. The Index Number consists of the first thirteen figures from the margin (primary characteristics) copied down in the order of their occurrence in the margin, placing a dash wherever a heavy rule occurs in the margin. Thus, *B. coli* belongs to the group 5312–41220–1111.

CHAPTER III

THE STRUCTURE AND MODE OF DEVELOPMENT OF BACTERIA—THE COMPOSITION OF BACTERIA

Bacteria are the smallest and the simplest forms of plant life known. Unlike the higher animals and plants, the entire organism consists of but a single cell. Individual cells differ in size, shape, method of cell-division, spore-formation, and the like; these features can be determined only by the use of high magnification. "Colonies" or masses of cells that develop upon certain food-substances often present definite peculiarities of form, color, consistency, and luster, which are apparent upon examination with simple lenses or with the naked eye. Similar differences may be observed among masses of larger objects: a grove of oak trees viewed from a distance too great to permit identification of the individual trees will still appear unlike a grove of pine trees. It is hence sometimes thought desirable to consider the morphology of bacteria under two heads: (*A*) the morphology of individual cells; (*B*) the morphology of masses of cells.

(A) The Morphology of Individual Cells

Dimensions.—Different kinds of bacteria vary materially in size. The average bacterium of rod shape measures about 2μ in length and 0.5μ in diameter (1μ = 1 micron or micromillimeter = $\frac{1}{1000}$ mm. = about $\frac{1}{25,000}$ inch). One large spherical bacterium that has been described measures about 2μ in diameter; the most common microbe found in suppurative processes is a spherical bacterium about 0.8μ in diameter. Considerable variation can occur within a single species. The bacillus of typhoid fever is found to range from 1μ to 3μ in length, even when the descendants of a single cell living under substantially identical conditions are examined. The largest bacteria belong, as a rule, to the group of spirally twisted or screw-shaped forms;[1] one of these[2] has been found to

[1] A bacillus (B. bütschlii), however, studied by Schaudinn (Arch. f. Protistenk., 1902, 1, p. 306) measures from 50 to 60μ in length and from 4 to 5μ in width.

[2] Spirillum colossum (Centralbl. f. Bakt., 1902, Abt. II, 9, p. 608).

measure as much as 3.5μ in diameter. Perhaps the largest pathogenic bacterium is the spirillum or spirochete of relapsing fever, which may measure up to 40μ in length.

One of the smallest of the well-known pathogenic forms is the so-called influenza bacillus (about 0.5μ × 0.2μ). Other micro-organisms, not surely known to be bacteria, are even smaller. The germs causing pleuropneumonia in cattle are so minute as to appear like mere points when viewed with a magnification of 2000 to 3000 diameters. The germ of foot-and-mouth disease will pass through the pores of the finest Berkefeld filter, and is so small as to be invisible under the highest lenses; but it can be cultivated by the usual laboratory procedures and its presence can be demonstrated by inoculation into susceptible animals. It is possible that other diseases, the causes of which are at present unknown, will be found to be due to ultramicroscopic organisms. It has been shown, for example, by Reed and Carroll[1] that the virus of yellow fever will pass through the pores of a compact porcelain filter. Special methods, such as microphotography by the ultra-violet light-rays[2] and illumination of a dark field, the "ultramicroscope" (p. 601) of Siedentopf and Szigmondy,[3] have been employed in the hope of rendering visible ultramicroscopic forms of life. Up to the present, however, these methods have not been successful in revealing the existence of hitherto unknown pathogenic micro-organisms.

Fig. 13.—Comparative size of human red blood-corpuscle, 6.9μ; typhoid bacillus, 2.4μ × 0.5μ; and influenza bacillus, 0.5μ × 0.2μ.

The mitochondria of living cells have been regarded by some investigators as symbiotic bacteria, but microchemical and physical differences exist which render such a supposition unlikely.[4]

Bacterial Forms and Their Interpretation.—For a long time the forms of bacteria were regarded as very simple and as comprising only three principal types, the sphere, the rod and the spiral (Fig. 14). Three chief divisions of bacteria, the coccus, bacillus and spirillum, were indeed based on this simple distinction. It is now known that a much wider range of variation occurs than was recognized by the

[1] Reed and Carroll: Amer. Med., 1902, 3, p. 301.
[2] Köhler: Ztschr. f. wiss. Mikrosc., 1904, 21, p. 129.
[3] Siedentopf and Szigmondy: Berl. klin. Wochensch., 1904, 41, p. 862.
[4] Cowdry and Olitsky: Jour. Exper. Med., 1922, 36, p. 521.

early bacteriologists, who were so influenced by the importance justly attached to working with pure cultures that they were inclined to regard unusual forms as contaminants. Much evidence has accumulated in favor of considerable variability in form.[1] One and the same microbe may show widely varying forms under different conditions of growth, as illustrated in the case of so well-known an organism as the cholera vibrio (Fig. 15). The diphtheria bacillus is another organism often developing unusual or "aberrant" forms. While a considerable degree of pleomorphism is thus well established there is difference of opinion as to its interpretation. Some of the most careful studies that have been made indicate a certain sequence in form development, the predominating forms in young cultures, 24 to 48 hours old, being different from those in older cultures. The irregular, bizarre forms found in old cultures were long characterized as *involution* or *degeneration* forms (Fig. 16) and were looked upon as abnormal. At the present day they are regarded by many bacteriologists as representing stages in an orderly progressive development towards "old age."[2] Some observers would go further and consider that bacterial growth phases give evidence of complicated life cycles, into which enter budding, cell conjugation, production of minute filterable forms and other phenomena. Difficulties connected with the smallness of bacteria, and the almost impossible task of following for any considerable period the descendants of a single cell have thus far prevented the general acceptance of this view. Evidence in favor of the occurrence of life cycles, especially in certain groups, is, however, strong, and it seems probable that future investigations will emphasize the complexity of bacteria rather than their simplicity.

Fig. 14.—Forms of bacteria.

The Finer Structure of Bacteria.—In spite of great technical difficulties some definite structural features can be made out. Many bacteria, perhaps all, are provided with a *capsule*, which originates from the outer layer of the cell-membrane; in stained preparations

[1] *E. g.* Löhnis: Mem. Nat. Acad. Sci., 1921, 16; Mellon: Jour. Bact., 1919, 4, p. 505; Henrici: Jour. Infect. Dis., 1925, 37, p. 75

[2] Cf. Clark and Ruehl: Jour. Bact. 1919, 4, p. 615.

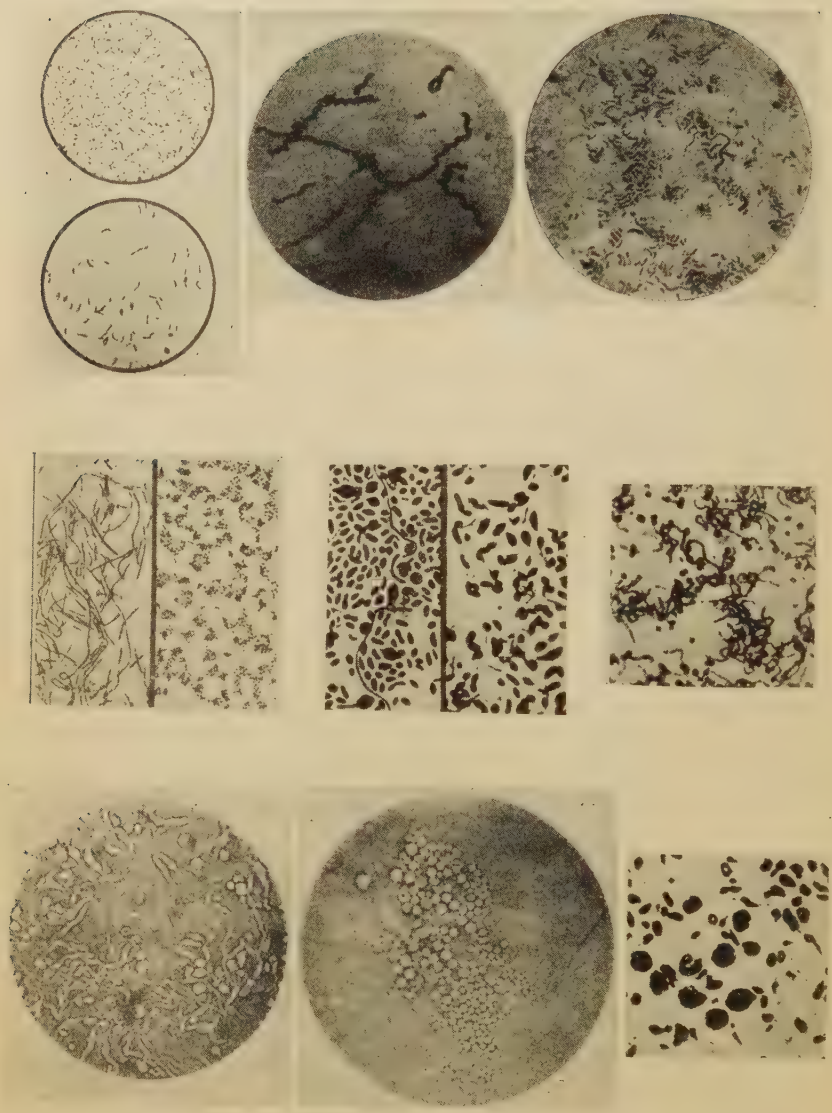


Fig. 15.—Cholera vibrio under various conditions of growth, as shown by six investigators (Henrici).

it can sometimes be seen surrounding the cell like a halo. The possession of capsular substance is believed by many investigators to be an attribute of all kinds of bacteria, although the substance is much more highly developed in some forms than in others. Certain organisms in which it is particularly conspicuous are commonly called the capsulated bacteria. Slimy cultures, such as those of B.

Fig. 16.—Involution forms of bacteria (enlarged about 1000): 1, Bacillus proteus mirabilis (Hauser); 2, Bact. aceticus (Hauser); 3, spirilla form of Bacillus anthracis (Petruschky); 4, Bact. halophilus (Russell); 5, Spirillum choleræ (van Ermengem).

mucosus capsulatus (p. 314), characterize the capsulated forms. The capsule is generally demonstrated most easily in preparations made directly from animal tissues, as is notably the case with the micrococcus of pneumonia, but it can also be seen in specimens from ordinary cultures, if appropriate methods are used.[1] Capsular substance is often formed abundantly in milk cultures. Buerger[2] finds that the presence of serous fluid in culture-media favors the development of capsules and that the capsules of the pneumo-

[1] Boni: Centralbl. f. Bakt., 1900, 28, p. 705.

[2] Buerger: Jour. Infect. Dis., 1907, 4, p. 426.

coccus can be readily demonstrated in preparations made from cultures on glucose-serum-agar. There is reason to believe that the capsular substance is chiefly carbohydrate in nature (polysaccharide) and that it varies significantly in its chemical composition in different kinds of bacteria. Its presence and abundance are in some way connected with the virulence or invasive power of micro-organisms (see pp. 190, 230).

The *cell-membrane* is chiefly remarkable for its chemical composition, since it differs from the cell-membrane of the higher plants in not containing cellulose. By many writers the membrane is regarded merely as the slightly differentiated outer portion of the cell-substance, and as deserving the name ectoplasm rather than cell-membrane. Its chemical character indicates its close relationship to the living protoplasm of the cell, as does also the fact that the flagella, or organs of locomotion, probably take their rise from this layer.

Fig. 17.—Chromatin granules in Bacillus megatherium (after Zettnow).

The nature of the *cell-substance* or entoplasm of bacteria has been the subject of much controversy. Especially have questions as to the character, disposition, and even the existence of nuclear material (chromatin) given occasion for many differences of opinion. The fact that the cell stains uniformly by ordinary methods has led, on the one hand, to the view that a bacterium is composed of cytoplasmic material without any nucleus, and, on the other hand, to the opposite opinion, that it is composed altogether or almost entirely of nuclear matter (chromatin), with possibly a thin outer envelop of cytoplasm. The latter view is supported by the great affinity shown by the bacterial cell-substance for the ordinary nuclear stains as well as by the capacity of the cell for very rapid cell-division. Considerable biologic significance attaches to the structure of cells so simple and presumably so primitive as bacteria, and the questions concerned have aroused widespread interest. Perhaps the most satisfactory view is that advanced by Zettnow,[1] which is based largely upon his own researches, especially upon some

[1] Zettnow: Zeitschr. f. Hyg., 1899, 30, p. 11.

remarkable observations on large spirilla which he succeeded in staining in a living and even motile condition, thus avoiding the production of artificial changes. Zettnow regards the cell-body of bacteria as composed largely, and in the case of certain vibrios almost wholly, of chromatin mingled with varying amounts of cytoplasm, a view not unlike that first advanced by Bütschli.[1] In many cases the chromatin, instead of being gathered together in a fairly compact mass or definite nucleus, is fragmented and distributed irregularly through the body of the cell (Fig. 17). In others a formed nucleus has been demonstrated, particularly in stages just preceding cell division. On the whole, the researches of Zettnow, A. Meyer, and others make it fairly certain that bacteria contain both chromatin and cytoplasm, and that the chromatin is present in great abundance, but varies in amount and in position in different cells.

When certain kinds of bacteria are treated with methylene-blue, various granules in the cell are observed to stain differently from the substance of the cell-body, for example, red against a blue background. These are the so-called *metachromatic granules*, about the nature of which opinion is still at variance. These granules are sometimes scattered through the cell-substance, sometimes massed at either pole, where they constitute the "polar granules" observed in the plague bacillus, the glanders bacillus, and other bacteria. In certain species the metachromatic granules are particularly easy to demonstrate, and their abundance may even constitute a character of some differential value (diphtheria bacillus). It seems probable that substances of radically diverse physiologic significance have been classed as metachromatic granules. In some instances such granules are simple degeneration products; in others they doubtless bear some important relation to the physiologic activities of the cell. They have even been compared, although on insufficient grounds, to the centrosomes of more highly specialized cells. It is more likely that they are in large part reserve food-substance. A. Meyer[2] has shown microchemically that some of the granules are fat, some glycogen, others a lecithin-like substance, and still others a peculiar protein-like compound. Some observers have noted that the metachromatic granules decrease in numbers during the period of

[1] Bütschli: "Ueber den Bau der Bakterien," Leipzig, 1890.
[2] Meyer, A.: Centralbl. f. Bakt., Abt. II, 1900, 6, p. 339.

active cell division and increase in the resting stage. The view advanced by a few writers, that some relation exists between the virulence of a culture and the richness of the cell in granules, has not been established.

Motility and the Organs of Locomotion.—Many kinds of bacteria are observed to be motile under the conditions in which bacteria are usually studied. Some of those forms in which motion has never been observed may perhaps possess the power of locomotion under certain unusual conditions. Independent bacterial motion is a true movement of translation, and is to be distinguished from the oscillating or quivering movement exhibited by all very minute

Figs. 18 and 19.—Flagella; Proteus vulgaris and large spirillum belonging to the group of sulfur bacteria (Zettnow).

particles suspended in water or other suitable fluids. The latter movement is the so-called "Brownian movement." This is a purely physical phenomenon due to so-called molecular bombardment, not to the activities of the living cell. Both dead bacilli and living non-motile bacilli show the Brownian movement. In particular cases where motility is sluggish it is often difficult to determine whether the changes in position that are observed are independent or are simply manifestations of the Brownian movement. Many bacteria are found to be motile when they are examined after removal from certain culture-media, but non-motile if they have been grown on other substances. One of the familiar instances of this sort is the case of the colon bacillus, which is motile when examined from young colonies on gelatin or agar, but is frequently non-motile when taken from broth. Conflicting statements concerning the motility of an organism often depend upon the fact that observations have not been made under comparable circumstances.

The rate at which a bacterium moves has been approximately measured. The typhoid bacillus may travel a distance of 4 mm., or about 2000 times its own length, in one hour; the cholera spirillum may attain for short distances a speed of 18 cm. per hour.

The power of locomotion in bacteria depends upon the possession of *flagella*, long, fragile, filamentous appendages which originate from the capsule, and by virtue of their power of contractility drive the bacterium through the water. Flagella have been seen in the living, unstained cell (large spirilla) by some observers, but ordinarily special methods must be applied to reveal their presence (p. 54). Abnormal bacterial flagella may be mistaken for spirochetes even by experienced observers.[1] Differences exist in respect to the position of the flagella on the cell-body: some forms possess only a single flagellum at one pole (*monotricha*, cholera spirillum); others possess a flagellum at each pole (*amphitricha*, many spirilla); others possess a tuft of flagella at one pole (*lophotricha*, certain large spirilla); and others have flagella projecting from the whole body of the cell, from the sides as well as the poles (*peritricha*, typhoid bacillus and many others (Figs. 18, 19). In some non-motile bacteria no flagella have ever been observed (*atricha*, anthrax bacillus). The number of flagella on the body of peritrichous bacteria varies considerably. Even closely allied bacterial species may differ in respect to the number of flagella they possess. The typhoid bacillus, for instance, possesses, as a rule, more flagella (ten to twelve) than the colon bacillus (two to six). The majority of actively motile bacteria belong either to the bacilli or the spirilla; very few micrococci are motile under ordinary conditions,[2] and no motile trichomycetes have been described.

Growth and Cell-division.—A bacterium can increase in size up to a certain point; the maximum size attainable, as among the higher forms of life, is singularly constant for each species. When the maximum is reached, cell-division may occur by simple partition or fission, dividing the cell into approximately equal halves. Division of the nuclear substance precedes that of the cell-body (Nakanishi[3]). Among bacilli and spirilla cell-division usually takes place

[1] Noguchi: Jour. Am. Med. Assn., 1926, 86, p. 1327.

[2] According to the investigations of Ellis (Centralbl. f. Bakt., Abt. II, 1902, 9, p. 546), all forms of micrococci possess flagella and are motile under favorable conditions. This assertion has not been confirmed.

[3] Nakanishi: Centralbl. f. Bakt., I, 1901, 30, pp. 97, 145, 193, 225.

at right angles to the long axis of the cell; among the cocci division may occur in three ways: in one plane only, resulting in the formation of chains (*streptococci*); or in two planes, giving rise to flat sheets of cells or irregular masses (*staphylococci*); or in three planes, producing cubical bales or packets (*sarcinæ*).[1] After cell-division the cells may remain connected (*streptobacilli* or *streptococci*) or they may become speedily disunited. Bacilli and spirilla show some elongation before division; cocci, as a rule, do not, although some cocci exhibit an increase in the diameter of the cell without any alteration of its spherical form.

While bacterial multiplication is commonly observed to take place by simple transverse fission, other modes of cell division may occur such as budding or Y-like splitting. It is possible that this unusual form of multiplication betokens the existence of more complicated life histories among bacteria than ordinarily assumed.

Under favorable conditions cell-division may take place quite rapidly (cholera vibrio, twenty minutes; hay bacillus, thirty minutes). Such rapidity of cell-division is sometimes referred to as if it were a peculiar quality of bacteria, but as a matter of fact the embryonic cells of many higher forms of life divide quite as rapidly as bacteria.

The remarkable thing about bacterial cell-division is not so much the rapidity with which one cell-division succeeds another, as the fact that a very short time suffices for the growth of the young cell to maturity. A young bacterial cell attains full size and acquires the capacity to produce in its turn an independent organism much sooner than most other forms of life. This rapid reproduction of distinct individuals is plainly different from the multiplication of embryonic cells among higher organisms. The rate of multiplication among the more complicated protozoa, which are also one-celled organisms, is considerably less rapid. Calkins[2] has shown that Paramœcium divides about once or twice in twenty-four hours. It has been estimated that if bacterial multiplication went on unchecked, and the division of each cell took place as often as once an hour, the descendants of each individual would in two days

[1] It may be noted that when the cells become separated after division, and change their position, it is difficult, if not impossible, to trace the direction of the division plane. Some of the organisms classed as staphylococci are said to be able to divide in three planes. (Fischer: "Structure and Function of Bacteria," tr. Oxford, 1900, p. 19.)

[2] Calkins: Archiv f. Entwickelungsmech., 1902, 15, p. 139.

number 281,500,000,000, and that in three days the progeny of a single cell would balance 148,356 hundredweight! No living organism, however, as was pointed out long ago by Darwin, can increase in exact geometric progression, for various checks and hindrances are always placed upon its multiplication by natural causes. In the case of bacteria a potent influence that tends to prevent unlimited multiplication is found in the interference with growth caused by the substances produced by bacteria themselves. Acids and other injurious products are commonly formed by bacteria during the disintegration of their food-substances, and accumulate in the immediate surroundings of the organisms, where they often inhibit all further multiplication. This is undoubtedly one of the ways in which bacterial growth is checked, although other factors, such as insufficient food, lack of moisture, unsuitable temperature, and the competition of other kinds of bacteria, also play a part.

Spore-formation.—The true *spores* or *endospores* of bacteria resist a heat of from 70° to 100° C., and are characterized by definite structural and physiologic qualities. In shape they are approximately spherical or oval. Spores are gifted, as a rule, with a very much higher resistance to all sorts of injurious influences than are the vegetative cells from which they spring. In addition to their great resistance to high temperatures, to the action of poisons and the like, they stain with great difficulty, these characteristics being probably due to their extraordinarily dense and compact structure. The highly refractive character of the unstained spore also is connected with the concentrated character of the spore substance.

An assembling or concentration of the nuclear material seems to precede spore-formation in some cases and constitutes the spore primordium. As a rule, a single cell forms only one spore; exceptions to this are very rare. Spore-formation among bacteria, therefore, is not a reproductive device for multiplying the number of individuals of the species, but more probably signifies the assumption of a resistant stage for the purpose of meeting the advent of unfavorable conditions of life.

The spore may be formed in any part of the cell, its position being generally constant in the same species. In some cases it does not exceed the diameter of the parent cell (anthrax bacillus); in others it may cause a bulging out of the wall of the cell at the

point where it lies (Fig. 20). If a swollen spore is formed at one pole, a "drumstick" appearance (tetanus bacillus) may result, or if it lies centrally the cell will become "spindle-shaped," a form to which the name clostridium has been given.

The spore of the anthrax bacillus, when brought under favorable conditions, first shows a change in the refractive property of the spore-substance; this is followed by a slight elongation of the spore, with a final bursting through of the spore-membrane and the outgrowth of a short rod, which then divides in the usual manner. The new outgrowth of the anthrax bacillus takes place at the pole of the spore; in the closely related hay bacillus it is at the equator. Other forms of bacteria exhibit intermediate methods of germination, and irregularities sometimes occur in the development of spores of the same species.

Fig. 20.—Spores, bacillus of symptomatic anthrax. Methylene-blue stain (Kolle and Wassermann).

Spore-formation is not very common among bacteria. It is most frequently observed in bacilli, less commonly in spirilla, and very rarely in micrococci. A noteworthy correlation of characters is shown in the almost unfailing occurrence of spore-formation in strict anaërobes (p. 82). Spore-forming aërobic bacteria are relatively less abundant. The spore-forming bacteria of known pathogenicity for man—certain anaërobic bacilli (bacillus of tetanus, of malignant edema, and a few others) and the anthrax bacillus—are few, a fortunate circumstance that materially facilitates and simplifies the disinfection and treatment of infectious diseases.

The conditions under which bacteria form spores vary with the nature of the organism. The bacillus of anthrax, as a rule, forms spores only when in contact with free oxygen, a fact that has a direct practical bearing upon the mode of disposal of the carcasses of cattle dying from this disease. The tetanus bacillus, on the other hand and the anaërobes in general, form spores in the entire absence of oxygen. A suitable temperature is essential to the formation

of spores: the anthrax bacillus forms spores most abundantly at about 30° to 32° C., and will not produce spores below a temperature of about 12°, its optimum of vegetative multiplication being about 37°. Lack of food is apparently not an adequate stimulus to spore-formation. In all cases a period of uninterrupted vegetative multiplication precedes the appearance of spores, and the conditions necessary for the production of spores seem to arrive simultaneously for most of the cells in a culture. In at least some cases the cause of spore-formation is to be found in an accumulation of metabolic products in the culture; these may perhaps be acids, perhaps other injurious compounds.[1] It must be kept in mind that, although some bacteria do not form spores under the conditions in which they are observed, it does not follow that they may not form spores under other and more suitable conditions. It is not possible to imitate with precision the "natural" conditions of life for all micro-organisms, and there is no complete justification for the assumption that because an organism has never been observed to form spores it never does so.

Physiologically the spore is to be considered as a resting-stage. It serves to tide the species over a period of dryness, famine, or unsuitable temperature, and to preserve alive in a hostile environment a sufficient number of individuals until such time as favorable conditions recur. The spore stage is, in fact, physiologically analogous to the periods of hibernation or estivation among higher forms of life. In this resting state the living matter of the spore may remain dormant for years or even for decades.

(B) The Morphology of Masses of Cells

In the case of many species of bacteria a single cell, when planted in a favorable medium and allowed to develop under suitable conditions of moisture, temperature, and air-supply, will in a few hours or days develop a "colony" so large that it can be plainly seen by the naked eye. In some instances such masses of cells, especially when the growth occurs upon certain culture-media, possess salient peculiarities which are highly characteristic of the species. In others the differences between colonies of closely allied species are exceedingly subtle, and can be detected at times only by a trained eye. Growths upon the nutrient gelatin commonly

[1] Migula: "System der Bakterien," Jena, 1897, I, p. 177.

used as a culture-medium are especially characteristic; on this medium the morphologic appearance of a mass of typhoid bacilli, for example, is quite distinct from that of a mass of anthrax or of diphtheria bacilli. The differences between the gelatin colonies of closely related organisms are often, however, as in the case of some members of the colon-typhoid group, almost or quite inappreciable.

The character of colonies is often profoundly affected by the density and viscosity of the culture-medium (Whipple[1]) and by the physical conditions under which the colonies develop (Dunham[2]). Upon nutrient agar the morphology of bacterial colonies is less distinctive than upon gelatin; the colonies upon potato and other solid food-substances are, as a rule, still less characteristic.

Besides the shape, size, and general structure of bacterial colonies, color is sometimes of service in differential diagnosis. Certain species produce variously colored pigments which are more or less characteristic of the organism forming them. A common microbe of suppuration owes its name of the "golden pus coccus" to its production of a golden-yellow pigment. The bacillus of blue-green pus (B. pyocyaneus) is also conspicuous for its pigment. It must be remembered, however, that among bacterial colonies, as among living organisms generally, there is no quality so variable as color, and that, as a rule, implicit dependence cannot be placed upon the pigmentation of a bacterial colony even as a mark of varietal or racial difference.

As an aid in distinguishing different species it must be admitted that great stress should not be laid upon the morphology of masses of bacteria. The mass-morphology, like the individual morphology, is subject to wide variation under varying conditions of life, and can be regarded as only one item in the sum-total of characters that go to make up the concept of a bacterial species. As has been pointed out by Marshall Ward,[3] "the attempt to determine species of bacteria by ordinary macroscopic methods leads to difficulties of the same kind as would be met if we tried to differentiate species from the marks presented by masses of trees in forests from a distance—say, in a balloon. A forest of a given species of tree would appear very different at different seasons and according to its age, the kind of

[1] Whipple: Technology Quarterly, 1902, 15, p. 127.
[2] Dunham: Science, 1903, 17, p. 372.
[3] Ward: Proc. Roy. Soc., 1897, 61, p. 415.

soil, climate, and so on, and the treatment it had received previous to planting."

One interesting difference between colony types appears to be of considerable significance. In a number of bacterial groups it has been found that rough (R) and smooth (S) colonies will develop from a single apparently pure culture. Under ordinary conditions transfers from the S and R colonies respectively breed true. The rough and smooth strains differ significantly in the stability of suspensions in salt solution, in virulence and in the other important characteristics. This marked and fairly persistent association of colony characteristics with deep-seated physiological and biochemical qualities shows the value of close observation of colony type.[1]

The Chemical Composition of Bacteria

The bodies of bacteria contain from about 80 to 88 per cent. of water, the amount showing considerable variation and depending partly on the nature of the organism, partly on the culture-medium. The ash is largely phosphoric acid, the P_2O_5 content often reaching as high as half the total ash weight (tubercle bacillus, 55.23 per cent., de Schweinitz and Dorset[2]). Sulfur, potassium, chlorin, and calcium are also present in notable amounts, together with usually smaller quantities of magnesium, iron, silica, etc. Some forms of filamentous bacteria, found especially in sewage-polluted water, contain in their protoplasm granules of sulfur (Beggiatoa). Others have notable deposits of iron in the sheath that surrounds the rather large filaments (Crenothrix).

Among the bacteria, as in the lower fungi generally, cellulose is conspicuous by its absence; but another and somewhat similar carbohydrate, designated as hemicellulose, is often present in abundance. Starch-like substances, staining blue with iodin, are also observed. It is a peculiarly interesting fact that a substance closely related to *chitin*, if not identical with it, has been found in a number of bacteria. It has been noted that in many respects the bacteria resemble the lower animals in their chemical composition.

Characteristic nitrogenous compounds—namely, nuclein, hypoxanthin, guanin, and the nuclein bases, such as adenin—occur

[1] A comprehensive discussion of rough and smooth bacterial types with full bibliography has been given by Hadley (Jour. Infect. Dis., 1927, 40, p. 1).

[2] de Schweinitz and Dorset: Centralbl. f. Bakt., 1897, 22, p. 209.

practically constantly in bacteria. Regarding the nature of the true protein substances in bacteria little is known. Much attention has been paid to the toxic constituents of the bacterial cell, and these substances are referred to in another place. The diffusible products of bacteria are also considered elsewhere (p. 102).

CHAPTER IV

THE EFFECT OF PHYSICAL AND CHEMICAL AGENTS UPON BACTERIA

It is a well-known biologic fact that various physical and chemical agencies affect profoundly the vital phenomena of all living cells; physical and chemical factors determine inexorably whether a micro-organism shall thrive and multiply, whether it shall lead a merely dormant existence or shall altogether perish. Among the most important of the natural environmental influences are temperature, light, moisture, oxygen-supply, and food-supply.

Temperature Relations.—Many bacteria show great adaptability to temperature conditions. The hay bacillus (B. subtilis) is able to multiply at 6° C. and also at 50° C. Three temperature limits may be distinguished: a minimum, or the lowest point at which growth occurs; an optimum, or the temperature of most luxuriant growth; and a maximum, or the highest temperature at which growth can take place. The position of these three points differs greatly for different species. The minimum for some bacteria may lie above the maximum for others: B. thermophilus, a species found in soil and fermenting manure, will not grow in certain conditions below 42°;[1] B. tuberculosis will not grow above 42°, while B. phosphorescens will not grow above 37°. The optimum for B. phosphorescens is 20°, for the hay bacillus 30°, for B. tuberculosis 38°, and for B. thermophilus 63° to 70°. Some bacteria are able to multiply at or very near the freezing-point,[2] while others are said to be able to multiply at 75° to 77°. Setchell[3]

[1] It has been shown by Rabinowitsch (Zeit. f. Hyg., 1895, 20, p. 154) that while many of the thermophilic bacteria are able to grow only at temperatures above 50° when in contact with air, they are able under anaërobic conditions to grow at the ordinary incubator temperature (37.5°), or even as low as 34°. Such species appear adapted both to an anaërobic life in the animal body at about 37° and also to aërobic life at the high temperatures found in fermenting manure.

[2] Forster: Centralbl. f. Bakt., 1887, 2, p. 337; M. Müller: Arch. f. Hyg., 1903, 47, p. 127.

[3] Setchell: Science, 1903, 17, p. 934.

has found bacteria living in the water of hot springs at a temperature of 89° C.! The range of temperature within which growth can take place also varies greatly in different species. Bacteria that have become habituated to living in the mammalian body (*e. g.*, B. tuberculosis) have a much narrower range than those whose habitat is the outer world (B. subtilis). The optimum temperature for most of the bacteria pathogenic for man lies, as might be expected, in the neighborhood of the normal temperature of the human body (37° C.). The following table gives the approximate temperature relations for several species:

Bacterium.	Minimum.	Optimum.	Maximum.
B. phosphorescens	0.0	20.0	37.0
B. fluorescens, var. liquefaciens	5.0	24.0	38.0
B. subtilis	6.0	30.0	50.0
S. choleræ	8.0	37.0	40.0
B. anthracis	14.0	37.0	45.0
B. tuberculosis	29.0	38.0	42.0
B. fitzianus	....	40.0	45.0
B. thermophilus	42.0	63–70	72.0

The wide range here shown in respect to the maximum and minimum temperatures that permit growth is paralleled by the diversity in bacterial resistance to extreme temperatures. Spores are always much more resistant to heat than vegetative forms, and some species when in the spore-stage can withstand the temperature of boiling water for upward of sixteen hours. The vegetative forms of most bacteria, on the other hand, are killed at 55° to 58° C. by ten minutes' exposure in the presence of moisture. As is well known, dry heat is much less effective as a germicide than steam: in a dry atmosphere temperatures ranging from 140° to 180° C. must be employed to insure sterilization. If steam under pressure be used, as in the autoclave, exposure for fifteen minutes to a temperature of 125° C. is sufficient to destroy all known microbes. The difference between moist and dry heat doubtless depends upon the fact that the chemical or physical changes that cause the coagulation of protein or death of protoplasm take place, like such actions generally, more readily in the presence of water.

It must be remembered that the death of an organism from heat is determined by time of exposure as well as by temperature. The tubercle bacillus is killed by 30 minutes' exposure at 58° C.

(136° F.), 20 minutes' exposure at 59° C. and 2 minutes' at 65° C. Employing a constant for time exposure the "thermal death-point" has been determined with considerable precision for the common micro-organisms. The usual method consists in exposing, for ten minutes, a suspension of the organisms in broth or salt solution to the action of a given temperature. That temperature at which all the organisms are destroyed is said to be the thermal death-point for the species. These fatal temperatures are lower than is popularly supposed. The thermal death-point (ten minutes' exposure) for the cholera spirillum is 58° to 60° C.; for the anthrax bacillus, vegetative form, 60° C., spore, 100° C.; for the typhoid bacillus, 58° to 60° C. Under certain circumstances a bacillus may be protected by a deposit of protein so that the thermal death-point is apparently raised. It has been shown that while tubercle bacilli in suspension in milk are destroyed at 60° in ten minutes, the pellicle that forms on the surface of milk during exposure at 60° may contain living bacilli after sixty minutes.[1] It may be noted that the thermal death-point of those bacteria that are at all likely to be present in polluted water is low (57° to 60° C.), and since these micro-organisms do not form spores, the practice of simply bringing water to the boiling-point suffices to insure its safety for drinking purposes.

Bacteria are much less sensitive to low than to high temperatures. The common microbes of water and soil, and also typical pathogenic bacteria like the typhoid and diphtheria bacilli, have been exposed for some days to the temperature of liquid air (about −190° C.) without destroying their vitality or sensibly impairing their biologic qualities. Cultures of bacteria have even been exposed to the temperature of liquid hydrogen (about −250° C.)[2] with the same negative result. On the other hand, when bacteria are frozen in water during the formation of natural ice, the death-rate is high. The questions relating to the duration of life of bacteria in natural ice possess an important practical interest, and will be discussed elsewhere (p. 338).

Light.—That light affects the metabolism of the living cell is well known, and the various reactions to light that are exhibited by the higher organisms have been the subject of much investigation.

[1] Th. Smith: Jour. Exper. Med., 1899, 4, p. 217.

[2] This temperature is far below that at which any chemical reaction is known to take place, and is only about 23 degrees above the absolute zero point, a temperature at which, it is believed, molecular movement ceases.

In connection with the study of bacteria the germicidal influence of light has received most attention. Diffuse daylight has been found to exercise a hindering effect upon bacterial growth and metabolic activity. Direct sunlight is highly injurious to certain forms of bacterial life, many micro-organisms being killed almost instantly when exposed to the full action of the sun's rays. That the unfavorable influence of sunlight is not due to the heat-rays is shown by the use of a screen (alum solution) which intercepts the heat rays, but allows the germicidal rays to pass through.

Since light has no effect upon bacteria in a vacuum, it may be inferred that the changes brought about by light under ordinary conditions are primarily oxidation processes of a kind incompatible with the continued life of the cell.

The action of light on bacteria has been picturesquely shown by protecting certain portions of a plate seeded with bacteria, and allowing the rest of the plate to receive the full effect of the sun's rays. In properly handled plates colonies of bacteria will develop in the shaded portions, but no colonies will appear in the exposed portions (Fig. 21).

The bactericidal action of light is confined to the ultra-violet region of the spectrum, beginning at 350μ, and extending with increasing intensity to the shortest wave-lengths measurable with a quartz spectograph: 185.6μ[1] (Fig. 22). These limits coincide with the absorption of ultra-violet light by a bacterial emulsion. Ultraviolet rays have been utilized in special forms of apparatus for water sterilization.

The electric light exerts a germicidal influence similar to that of the sun's rays. The Röntgen rays have not yet been definitely shown to exert any germicidal effect.

Moisture.—Many of the higher forms of life display considerable resistance to drying. The small aquatic worms known as rotifers will revive after months or even years of prolonged desiccation. If, however, the actual body substance is not, like the rotifer, protected by a gelatinous capsule, the complete removal of moisture speedily destroys life. Even the seeds of the higher plants which are specially adapted for resistance to drying rarely outlast ten to twenty years.

[1] Bayne-Jones and Van der Lingen: Bull. Johns Hopkins Hosp., 1923, 34, p. 11.

Fig. 21.—An agar plate of anthrax spores exposed behind a stencil plate Y from 12.15 to 3.15 p. m. on March 27, and then incubated at 20°–22°C., and photographed at intervals. The photograph was taken as a transparency, against a N-window. The two crescentic areas are due to the agar-film not completely covering the plate in this case. Seventy-two hours' incubation (H. Marshall Ward).

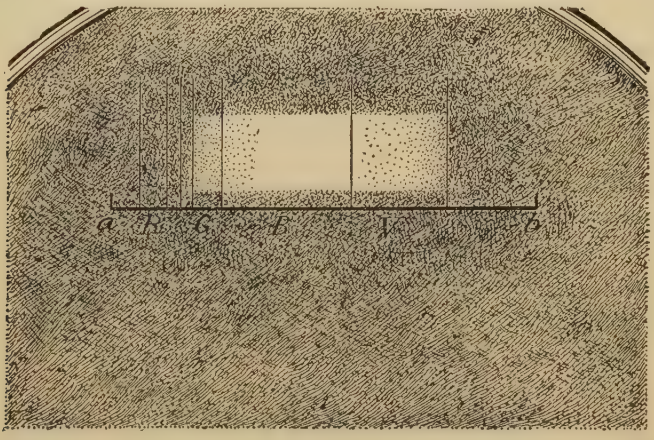


Fig. 22.—Bacterio-photograph of the spectrum (H. Marshall Ward). Agar plate of spores of Bacillus anthracis, exposed to the action of the solar spectrum for five hours on August 16th (11 a. m. to 4 p. m.). The spectrum was very bright, and nearly, though not quite, pure. The photograph shows the conditions of the plate after twenty-four hours' incubation at 25° C. This figure shows the limits of the various regions of the visible spectrum marked on it (*R*, red; *G*, green; *B*, blue; *V*, violet). The base line *a*—*b* shows the length of the slot through which the spectrum shone on the plate. All parts of the agar plate not exposed are rendered evenly opaque by the colonies germinated out from the spores; similarly the spores in the infra-red, red, orange-yellow are unhurt, as are those to the other end of the violet. The action of the light begins in the green-blue and attains its maximum in the blue-violet, near the Fraunhofer's line *G*.

Most of the vegetative forms of bacteria are rather quickly killed by ordinary air drying, although there are great differences among the different forms. The tubercle bacillus is one of the more resistant, the cholera spirillum one of the more sensitive, to drying. Exposure to desiccation for a few hours, or at most a few days, destroys the majority of known pathogenic microbes, so that infection through the air, except where floating bacteria are protected by their position within epithelial scales or in droplets of moisture, is not so common as popularly supposed.

The spores of bacteria are much more resistant to drying than the vegetative forms. The spores of the anthrax bacillus will germinate after remaining in a dry condition for at least ten years.

Oxygen Supply.—Bacteria may be divided into three classes with reference to their need for oxygen: the *obligate aërobes*, or those that require free oxygen for the maintenance of their life activities; the *obligate anaërobes*, or those that do not grow except in the almost complete absence of free oxygen; and the *facultative anaërobes*, or those that can thrive in either the presence or the absence of oxygen.

The *obligate aërobes* comprise many of the ordinary air and water bacteria, especially those of the pigment-forming varieties. Among pathogenic bacteria the diphtheria bacillus and the cholera spirillum are forms that require a supply of free oxygen. The different kinds of aërobic bacteria vary in respect to their optimal oxygen tension. This is beautifully illustrated by the "respiration figures" pictured by Beijerinck,[1] which show that different kinds of bacteria grow best at different levels in fluid media, the thickest swarm of each species being at that level at which the oxygen tension is most suitable. Wherry and Oliver[2] have made interesting observations tending to show that many endoparasites become adapted to a tension of oxygen below the atmospheric.

The discovery of an *obligate anaërobe* by Pasteur in 1861 was the cause of one of the most important changes wrought by bacteriology in the biologic conceptions then current. All of the organisms known up to that time required free oxygen to support life, and Pasteur's discovery was at first received with considerable incredulity. It has since been shown, however, by experiments of great

[1] Beijerinck: Centralbl. f. Bakt., Abt. I, 1893, 14, p. 837.

[2] Wherry and Oliver: Jour. Infect. Dis., 1916, 19, p. 288; 1917, 20, p. 28.

precision, that bacteria actually exist which are able to live and multiply in the almost complete absence of free oxygen, provided their food contains oxygen in suitable combinations. Anaërobes will grow in media where reduced hemoglobin remains unchanged and reduced methylene-blue shows no trace of reoxidation. The practical absence of oxygen is further demonstrated by the fact that strictly aërobic bacteria are not able to grow at all under these conditions. It has been found, however, that conditions for the growth even of the obligate anaërobes are not afforded by the entire absence of oxygen, but by the presence of very minute quantities of oxygen, which, according to Chudiakow,[1] are utilized by the anaërobes in their metabolic activities. Certain anaërobes, furthermore, can become acclimated to growth in gradually rising amounts of oxygen until the original oxygen limit is greatly exceeded.

It is found experimentally that anaërobic bacteria as a class thrive best in the presence of substances capable of undergoing reduction or fermentation.[2] The peculiar phenomenon of anaërobiosis may perhaps be explained by supposing that anaërobes are bacteria specially qualified to obtain their needed energy from the simple splitting up of organic compounds without oxidation. Although it is true that, in its original form, Pasteur's conception of fermentation as "life without air" is no longer tenable, it cannot be questioned that in many cases anaërobic life is conditioned by the ability of an organism to ferment certain organic compounds. In a modified sense, Pasteur's explanation of fermentation as due to the adjustment of certain micro-organisms to an anaërobic mode of life affords a satisfactory view of the biologic significance of anaërobiosis. In other words, if a microbe is able to obtain the energy necessary for its life activities by reducing processes without resorting to processes of oxidation it can live an anaërobic life; if it is so addicted to an anaërobic mode of life that the presence of oxygen, except in minimal quantities, interferes with its habitual methods of attacking food-substances, it is an obligate anaërobe; if, on the other hand, it can obtain energy only through the direct oxidation

[1] Chudiakow: Centralbl. f. Bakt., 1898, Abt. II, 4, p. 389.

[2] It is a common observation that many bacteria will not grow up into the closed arm of the fermentation tube except when the culture-medium contains certain sugars or other fermentable substances. The presence of certain aërobic bacteria or their products, or the products of anaërobic bacteria, also permits growth in the closed arm.

of organic substances, it is an obligate aërobe. Novy has suggested that the difference between obligate aërobes and anaërobes finds its basis in the nature of the enzymes present, one enzyme being activated by pure oxygen, while the other is able to act only when oxygen is absent.

The whole question of the respiration of microbes has been studied especially by Novy and his associates.[1] Oxygen is consumed and carbon dioxid given off during growth, as with higher forms of life. For the tubercle bacillus Novy and Soule found the optional concentration of O_2 to be about 40–50 per cent.; a fair growth of the tubercle bacillus was obtained in 90 per cent. CO_2, but these authors do not believe that CO_2 is utilized by the tubercle bacillus or other bacteria. Rockwell and Highberger, however, consider that carbon dioxide is, in some way, a vital factor in growth.[2]

Food-supply.—Bacteria are able to satisfy their food requirements upon the most diverse substances. Organic compounds in great variety can serve as food. Complex nitrogenous bodies, especially, which contain a large amount of available potential energy, are attacked eagerly by many species, as witnessed in the familiar phenomena of decay and decomposition. Less complex molecules can also serve as a source of energy. Many bacteria, including pathogenic forms, such as the cholera spirillum and others, will grow upon non-protein media which consist of a solution in distilled water of simple mineral phosphates and sulfates together with asparagin or ammonium salts of the organic acids (succinic, lactic, citric). Particular interest attaches to the ability of certain micro-organisms to construct their living substance wholly out of inorganic compounds. It has long been known that organisms containing chlorophyl or allied pigments are able, with the aid of the sunlight, to effect such a synthesis, but it was supposed that a sharp distinction must be drawn between the chlorophyl-bearing and the non-chlorophylaceous organisms. The ability of the so-called nitrifying organisms (Winogradsky) to develop in the presence of very simple mineral salts, and in the entire absence of organic matter of any kind, has completely overthrown this distinction (Ch. XXXIV). By these organisms the energy necessary for development is obtained from the oxidation of very slightly energized compounds

[1] Novy et al: Jour. Infect. Dis., 1925, 36, pp. 109, 168, 245, 343.
[2] Rockwell and Highberger: Jour. Infect. Dis., 1926, 38, p. 92.

like the mineral ammonium salts and even nitrites. Since organic carbon compounds are also formed by the nitrifying bacteria, it follows that *a complete synthesis of organic matter is effected by these organisms independently of the presence of pigment and the action of the sun's rays.* The nitrifying organisms, some of which are able to oxidize ammonia to nitrites, and others to oxidize nitrites to nitrates, are so wedded to their particular modes of metabolic activity that they are quite unable to thrive in the presence of organic substances, a condition analogous to that presented by the obligate anaërobes. Beijerinck and Van Delden[1] have reported the discovery of a remarkable organism, B. oligocarbophilus, which is capable of growing in water containing merely nitrates and other fully mineralized salts, and is able to utilize in its development a volatile carbon compound of unknown nature which is present as an impurity in ordinary atmospheric air. In association with certain other bacteria it can oxidize hydrogen, perhaps according to the equation:

$$CO_2 + H_2O + H_2 = CO + 2H_2O.$$

Pure cultures of B. oligocarbophilus are capable of oxidizing and assimilating carbon monoxid!

According to Kaserer,[2] B. pantotrophus, by a somewhat similar process, generates formaldehyd, which it can then use as a nutritive substance.

$$CO_2 + H_2O + 2H_2 = HCHO + 2H_2O.$$

This organism is able to endure the presence of formaldehyd in a strength of 1 : 15,000.

Bacteria are frequently distinguished as *saprophytes* and *parasites*, on the basis of their source of food-supply. Saprophytes are those forms able to obtain the requisite energy for growth from dead or lifeless matter; parasites are able to thrive within or upon the living substance of various animal or plant hosts. Many parasitic organisms are able to lead also a saprophytic existence, as shown by the ability of the tubercle bacillus, the plague bacillus, and many others, to grow not only in the human body, but also upon the ordinary culture-media employed in bacteriologic laboratories. A few parasitic forms, however, are so highly specialized for a life in contact with living tissues, and even the tissues of a particular host, that they are unable to grow under any other circumstances. This

[1] Beijerinck and Van Delden: Centralbl. f. Bakt., Abt. II, 1903, 10, p. 33.

[2] Kaserer: Centralbl. f. Bakt., 1906, 16, p. 681.

appears, for instance, to be the case with the bacillus of leprosy, which is able to grow in the body of man and in apes, but resists attempts to cultivate it on lifeless food-substances or in the bodies of other animals. On the other hand, most of the ordinary water and soil bacteria are powerless to grow when introduced into the animal body, and seem entirely unadapted to a parasitic mode of life.

The *concentration* and *reaction* of a nutrient substance are factors of some importance. In general, organic substances in solution are available as sources of food-supply for bacteria only when in certain degrees of dilution. A familiar instance is the speedy souring of a dilute sugar solution as contrasted with the keeping qualities of a thick syrup. The osmotic adjustment required of a bacterial cell suddenly introduced into a concentrated fluid is too great to be readily compassed.

Most pathogenic bacteria thrive best in a food-medium that reacts neutral or slightly acid to phenolphthalein. Different species, however, behave differently. The cholera spirillum is quite sensitive to the presence of a very small amount of acid, while the typhoid bacillus is not checked by a distinct acid reaction. Most bacteria found in water grow best in a medium with a hydrogen ion concentration between $P_H 6.2$ and $P_H 7.0$.

The importance of minute quantities of certain organic substances of unknown nature (*vitamins*) in the nutrition of the higher animals has led to inquiry whether similar accessory substances are of significance in bacterial growth. Many bacteria are able to multiply and give rise to their characteristic products in simple non-protein media so that the presence of organic substances does not seem essential. On the other hand, the presence of organic extracts of various kinds favors the growth of many micro-organisms. The growth of Pfeiffer's influenza bacillus has been definitely shown to be dependent upon the presence of a heat-resistant "X" factor and a heat-sensitive "V" factor, both present in blood and also in certain plant tissues. The same organism is favorably influenced by the products of growth of other microbes.

Other Environmental Influences.—Among other factors which have been studied with reference to their effect upon bacteria are atmospheric pressure, mechanical agitation, and electricity.

Pressures of 600 to 700 atmospheres are said by some observers to have an inhibitory effect upon putrefactive processes, but, on the

other hand, others state that living micro-organisms are not affected by exposure for twenty-four hours to a pressure of 600 atmospheres. According to Roger[1] a pressure of 2000 atmospheres lessens the virulence of the anthrax bacillus. Higher pressures destroy bacteria. Non-spore-forming bacteria are killed in fourteen hours by a direct pressure of 6000 atmospheres, and spores by about 12,000 atmospheres. Non-spore-bearing bacteria are killed by CO_2 of 50 atmospheres pressure in about one and one-half hours, while nitrogen of 120 atmospheres has no effect.[2]

The evidence in respect to the influence of *mechanical agitation* upon the life of bacteria is somewhat conflicting, but, on the whole, indicates that prolonged shaking, whether moderate or violent, of a fluid containing bacteria has little, if any, influence. Shaking will, however, sometimes cause the separation of loosely cohering cells, and this may lead to a simulation of cell multiplication, if dependence be placed on a count of bacterial colonies. When glass pearls or similar objects are shaken up together with bacteria, the organisms are mechanically injured by the successive shocks and destroyed.

Experiments made to determine the effect of the *electric current* upon bacteria have been in too many cases conducted loosely, and inferences have been drawn that have not been warranted by the conditions of the experiment. In some instances when a small amount of fluid is used, a rise in temperature is produced which is sufficient to account for the death of bacteria; in other cases death is due to the action of strongly germicidal substances, like chlorin and ozone, which are liberated by the passage of the electric current. When the effects due to heat and to the electrolytic production of germicides are eliminated, it is very doubtful whether any direct germicidal action can be properly attributed to the electric current. Under suitable conditions it can be shown that when bacteria are subjected to an electrical potential they move toward the *anode*. This is interpreted to imply that the bacteria are electro-negatively charged with respect to water. The charge can be increased or decreased and even reversed. These observations are being developed to account for the stability of bacterial suspensions and to explain certain phases of agglutination phenomena.[3]

[1] Roger: Arch. de Physiol., 1895, 5s., 7, p. 12.

[2] Larson, Hartzell, and Diehl: Jour. Infect. Dis., 1918, 22, p. 271.

[3] See, for example, Winslow, Falk, and Caulfeild: Jour. Gen. Physiol., 1923, 6, p. 177.

Adaptability of Bacteria to Varying Conditions of Life.—It has already been pointed out that different kinds of bacteria vary greatly in their response to different physical and chemical agencies. It is also a noteworthy fact that one and the same kind of microorganism is able to adapt itself to widely different conditions of life. Thus, Dieudonné[1] has shown that by cultivating the anthrax bacillus at gradually decreasing temperatures a degree of acclimatization to cold is finally attained by this organism which enables it to grow at a temperature as low as 10° C. The adaptability to changed conditions shown by the tubercle bacillus, which when first isolated from the mammalian body grows reluctantly on artificial media, but with continued cultivation becomes more saprophytic, is another case of the same order. It is probable that these adjustments to different conditions of life are in part due to the selective influences that are always at work when cultures of organisms, containing many individual cells, are exposed to a changed environment: that is, when the temperature of a culture is raised, certain cells, the least resistant, will be destroyed first, while the more resistant will survive and their descendants will inherit the resistant qualities of the parents; eventually the whole culture by this process of continued selection will come to possess a heightened tolerance of high temperatures. In addition to this factor, however, individual adaptation on the part of the protoplasm of the individual cell may occur also, as indicated by analogous experiments with other organisms, for example, by Dallinger's results with flagellates,[2] which he succeeded, in the course of several years, in rearing by slow stages up to a temperature of 70° C., when the experiment was ended by accident. At the beginning of the experiment the flagellates were killed at 23° C. Probably bacterial protoplasm likewise can become directly adjusted to changed conditions.

Effect of Chemical Substances upon Bacteria.—The phenomena of positive and negative chemotaxis are fully exemplified in bacterial life. It has been frequently demonstrated that bacteria, like other free-moving organisms, are apparently attracted by certain chemical substances in solution (positive chemotaxis) and repelled by others (negative chemotaxis). These movements are ordinarily regarded as the direct result of a chemical stimulus. According to the view

[1] Dieudonné: Arb. a. d. kaiserl. Ges., 1894, 9, p. 492.
[2] Dallinger: Jour. Roy. Mic. Soc., 1887, 1, p. 185.

held by Jennings,[1] the swarming of bacteria around algæ that are evolving oxygen, or around any other points where favorable nutrient conditions exist, is not to be looked upon as due to a definite attraction exerted upon the bacterial cell, but as caused simply by the tendency to remain at those points where the conditions are favorable. In the course of their aimless wanderings bacteria eventually arrive at those spots where conditions—as the oxygen tension—are highly suitable; there they remain.

The tendency of aërobic bacteria to collect near that portion of an algal filament where oxygen is being most abundantly evolved has been utilized by Engelmann in a beautiful experiment for showing the effect exerted upon assimilation by the different parts of the solar spectrum. The greatest aggregation of bacteria occurs

Fig. 23.—Bacteria gathered around alga evolving oxygen (from Engelmann). Piece of cladophora with swarming bacteria in the microspectrum (gaslight). The chlorophyll grains which fill the cells very uniformly are omitted, and, instead, the absorption band between *B* and *C* and the tolerably pronounced band at the violet end between *E* and *F*, are indicated by shading.

at the red end of the spectrum (Fig. 23), indicating that the maximum assimilative activity of the algal protoplasm is proceeding at this point.

Many bacteria in the course of their growth give rise to substances, such as acids, which are more or less injurious to cell life. The cessation of growth which takes place after a time in cultures of bacteria upon artificial culture-media is thought by some to be due not to the exhaustion of the available food-supply, but to the accumulation of metabolic products which interfere with bacterial

[1] Jennings, H. S.: "Behavior of the Lower Organisms," New York, 1906, p. 39.

development. Such substances, however, are probably not, as they are sometimes assumed to be, complex cellular products peculiar to the cell producing them, but are simple acids or other substances due to molecular disintegration. It is, to say the least, questionable whether specific inhibitory "autotoxins" are produced in bacterial cultures, although the matter needs further investigation.

In a state of nature single species or "pure cultures" of bacteria rarely have the field to themselves, the natural processes of decomposition and disintegration being carried on by a host of different microbes. Under open competition the growth of one species may be sometimes hindered, sometimes assisted, by products of associated or competing species. The favoring influence that aërobic bacteria and their products have upon the life of anaërobic bacteria has already been mentioned. The so-called "antagonism" between certain bacterial species is undoubtedly due to the nature of their chemical products. The presence of those microbes that produce acid by fermenting carbohydrates is, of course, peculiarly unfavorable to microbes sensitive to acid. The chemical products of bacterial activity will be considered more at length in the following chapter.

Disinfectants and Antiseptics.—Chemical substances have been extensively employed for antisepsis and disinfection. An enormous number of such substances have been advocated for various purposes, but in many cases perfectly satisfactory disinfection is obtained with familiar and relatively simple chemical compounds. Most proprietary disinfectants are disproportionally expensive, and, owing to the lack of precise information as to their composition and strength, relatively untrustworthy.

A distinction is commonly made between antiseptics and disinfectants. A disinfectant or germicide kills the microbes with which it comes in contact. An antiseptic substance is one that restrains or checks the development of bacteria, but does not destroy them. A substance may be only antiseptic in high dilution, but germicidal if more concentrated. For example, a 1 : 300,000 solution of corrosive sublimate will prevent the development of anthrax spores, but a 1 : 1000 solution is necessary to kill them. The brine that is used in pickling meat has a strongly antiseptic action, but pathogenic bacteria have been known to retain their vitality in salt meat

for long periods. Different methods and substances have been found adapted for different purposes.[1]

For the purpose of disinfecting rooms or apartments a gaseous substance is particularly useful. The custom of burning sulfur in infected rooms has the sanction of antiquity, and under certain conditions is reasonably effective. The sulfur dioxid (SO_2) that is formed when sulfur is burned is a germicide only in the presence of abundant moisture, sulfurous acid (H_2SO_3) being the active agent. Roughly speaking, about one-fifth of a pound of water should be volatilized for each pound of sulfur burned. "Exposure for eight hours to an atmosphere containing at least four volumes per cent. of this gas in the presence of moisture" is said to destroy all non-spore-bearing pathogenic bacteria. This requires the combustion of about 4 to 5 pounds of sulfur for every 1000 cubic feet. Not only bacteria, but mosquitos, fleas, and other possible insect carriers of pathogenic micro-organisms are destroyed, and this is one of the advantages of sulfur fumigation. The use of sulfur has the disadvantage that it lacks penetrative power and that it injures certain fabrics and materials. For the latter reason, especially, sulfur as a disinfectant of dwelling-houses has been largely superseded by formaldehyd (HCHO).

Formaldehyd, which is usually sold under the trade name of formalin, a 33 to 40 per cent. solution of the gas in water, is a more effective germicide than sulfur dioxid and has the great advantage that it does not damage books, paintings, and delicate fabrics, attack ordinary dyes, or act upon most metals. Like sulfur, it is efficacious only in the presence of moisture. According to McClintic[2] the humidity should not be lower than 60 per cent. and the temperature not less than 16° C. in order to obtain the best results. For practical purposes the gas may be generated in a variety of ways:

1. If the vapor of methyl alcohol be passed over a highly heated surface—as, for example, over asbestos discs coated with finely divided platinum—the partial oxidation that occurs gives rise to formaldehyd:

$$CH_3OH + O = HCHO + H_2O.$$

[1] The extensive use of heat for sterilizing or disinfecting instruments and surgical apparatus and for rendering bandages and dressings aseptic has been elsewhere considered.

[2] McClintic: Bull. 27, Hyg. Lab. and Mar. Hosp. Service, 1906.

On this principle a number of lamps have been devised that have been used to some extent, but considerations of economy and of ease and efficiency of application have prevented a very general introduction of this type of generator.

2. If formalin is simply boiled, two molecules of formaldehyd unite, and a polymer, paraformaldehyd, is formed. The first effect of heating formalin, therefore, is to drive off water with a relatively small admixture of formaldehyd gas. If evaporation is continued, the boiling-point of the solution is raised and a temperature reached at which the polymer is broken up and formaldehyd is disengaged. The same end is reached more expeditiously if the formalin is superheated either in an autoclave under pressure, or in other special forms of apparatus. When steam under pressure is used, calcium chlorid (30 per cent.) or some other neutral salt is added to the formalin to prevent polymerization (Trillat system). Great penetration may be assured by the use of formaldehyd and dry heat in a partial vacuum. Many of the pieces of apparatus designed for liberating formaldehyd from formalin by heat are reasonably effective, but are heavy and expensive. Simple heating of formalin in almost any kind of vessel will give good results if a liberal amount of formalin be used—that is, 12 ounces for each 1000 cubic feet—if some substance, as for example 10 per cent. of glycerol, which raises the boiling-point, be added, and if evaporation be not too rapid.

3. If the solid polymer of formaldehyd is heated, not ignited, formaldehyd is evolved. A lamp has been especially constructed for this purpose, and with the use of tablets or pastils of paraform affords an easy and effective means of disinfecting small rooms (Schering system).

4. Some formaldehyd is given off from the watery solution at ordinary room temperature. Use has been made of this in the method of spraying formalin upon sheets hung in a tightly sealed room. The gas, however, is evolved slowly under these conditions and in uncertain quantity, dependent upon many variables, such as temperature, amount of exposed surface, and other factors. Diffusion is necessarily poor.

5. Good results have been reported from the simple method of pouring formalin over crystals of potassium permanganate in an open vessel protected by some non-conductive material in such

a way as to retain the heat. Sixteen ounces of formalin and six and three-fourths ounces of permanganate are recommended as a suitable proportion at temperatures of 16° C. and over; larger quantities of formalin are necessary if the temperature is below 16° C.

The value of terminal disinfection after such diseases as diphtheria and scarlet fever is questioned by many health authorities. The terminal disinfection of rooms has been abandoned or greatly modified in several cities (Providence, New York, and Boston), and there has been no consequent increase in the prevalence of infectious disease. As a practical measure for the prevention of disease terminal disinfection is on trial.

The disinfection of dejecta, sputum, and similar substances suspected of harboring disease germs may be effectively carried out by cremation or by boiling; but in practice a great variety of chemical substances, the ordinary "disinfectants" of commerce, are employed for this purpose. It should be remembered that, in accord with the modern theory of solutions and electrolytic dissociation, many solutions are chemically and biologically potent in proportion to the number of free ions or dissociated fragments of molecules that they contain. A comparison of the disinfecting power of the various metallic salts, for example, on the basis of percentage solutions would be misleading, since the degree of dissociation would differ in the several cases. A gram-molecule or equimolecular solution must be employed in order to obtain comparable results. Unless dissociation takes place, solutions of metallic salts are practically without germicidal effect. A solution of mercuric chlorid in absolute alcohol has substantially no disinfecting power, but if water be added, the germicidal power of the solution increases proportionately to the amount of water added. Among the important points discovered by Krönig and Paul[1] in a now classic study of the action of disinfectants, it was shown that the disinfecting properties of the salt of a metal are due in large part to the specific action of the metallic ion, but also in some degree to the anion and to the undissociated part of the salt. The disinfecting power of an acid is approximately proportional to the hydrogen-ion concentration.[2]

[1] Krönig and Paul: Zeitschr. f. Hyg., 1897, 25, p. 1.
[2] Norton and Hsu: Jour. Infect. Dis., 1916, 18, p. 180.

The number of chemical compounds used or recommended for purposes of disinfection is legion. Many compounds which are used depend for their effect upon the action of freshly liberated or nascent oxygen. One of them is potassium permanganate, which forms the basis of many of the patent disinfectants, but is expensive in proportion to its efficiency. Ozone is a powerful germicide and has been used with success in sterilizing water on a large scale, but cannot be advantageously generated and applied for ordinary purposes.

Among the metallic salts used for disinfection, corrosive sublimate (mercuric chlorid, bichlorid of mercury, $HgCl_2$) is one of the best known and most effective. In the presence of considerable quantities of organic matter, however, its use is totally inadmissible, for the reason that inert combinations between the Hg-ions and certain albuminous substances are formed, and a large part of the mercury thus rendered unavailable for action upon bacteria. In alkaline fluids such as many of the body fluids and pathologic exudates, oxids or hydroxids of mercury may be precipitated out, but the addition of a small quantity of common salt (NaCl) will prevent this. Corrosive sublimate is particularly serviceable in a standard solution, 1 : 1000, for the disinfection of the hands and for washing woodwork, floors, and furniture. It must be kept in mind that corrosive sublimate attacks metal, and is hence inapplicable for the disinfection of instruments and for use in plumbing fixtures.

Among the metallic salts that have been rather extensively used for disinfection are ferrous sulfate (copperas), zinc chlorid, and copper sulfate. The two former substances are of feeble germicidal power and are of little practical value except as deodorants. Copper sulfate has in recent years won a deserved reputation for destroying the microscopic algæ that sometimes impart offensive odors and tastes to public water-supplies. The death of the algæ in large bodies of water may be effected by as great a dilution as 1 : 1,000,000. Copper sulfate possesses bactericidal as well as algacidal power; according to a number of observers a dilution of 1 : 400,000 will kill typhoid bacilli in twenty-four hours in water relatively free from organic matter but most investigators doubt its practical applicability for water disinfection on a large scale.

Some of the best-known and most efficient germicides are coal-tar products. Carbolic acid, or phenol (C_6H_5OH), is probably

still the most generally used disinfectant, although of late years it has been to some extent supplanted by similar organic substances. When used in strong enough solution (5 per cent.) it will destroy all vegetative bacteria and most spores, even in the presence of considerable organic matter. Another merit of carbolic acid is the comparative permanence of its solutions. Cresol [$C_6H_4(CH_3)OH$] is somewhat like carbolic acid in composition, and is present in large amount in "crude carbolic acid"; it is almost insoluble in water. Tricresol is a mixture of orthocresol, metacresol, and paracresol; it dissolves in water in a 2.5 per cent. solution, which is about three times as powerful as carbolic acid. Creolin and lysol contain large amounts of the cresols, and some phenol, mixed with soap, which greatly facilitates the solution of cresol. Many of the widely used coal-tar disinfectants are quite poisonous, and care should be taken to guard against accidental or suicidal poisoning. An effective liquid disinfectant, cheap and easily prepared, is recommended by the U. S. Public Health Service.[1] This so-called pine oil disinfectant is a by-product from the manufacture of wood turpentine. Its phenol coefficient is between 4 and 6, and the cost of preparation does not exceed 50 cents per gallon. It may be used wherever the ordinary coal-tar compounds are employed and possesses decided advantages over these substances.

The sterilization of feces is advantageously carried out by the use of calcium hydroxid ($Ca(OH)_2$). In laboratory experiments a 1 per cent. solution of freshly slaked lime in water has been found to kill nearly all pathogenic bacteria within a few hours. A 20 per cent. solution mixed with an equal part of the feces and urine of a typhoid patient will bring about complete disinfection within an hour. The cheapness and high efficiency of freshly slaked lime render it the most useful of the common disinfectants for bowel discharges, the contents of privy vaults, and manure piles. Air-slaked lime, calcium carbonate, has no antiseptic value. A mixture of chlorid and hypochlorite of lime, the "bleaching powder" of commerce, is when fresh also very efficacious through its oxidizing action. The use of substances, like carbolic acid and chlorid of lime, that possess a pronounced and lingering odor, is open to the objection that the mere presence of the odor engenders a false sense

[1] Stevenson: Public Health Reports, 1915, 30^2, p. 3004.

of safety, regardless of the strength of the germicide used and the duration of its application.

Whatever be the aim or method of disinfection, it must be remembered that simple cleanliness is an indispensable adjunct, and that the use of hot water and soapsuds or soda solution is a powerful aid to the removal and destruction of disease germs. The germicidal action of sunlight in the presence of abundant oxygen supply should be utilized whenever possible.

Recommended Procedures for Disinfection.—In the case of a patient suffering from infectious disease, different methods of disinfection are necessary according to the channel by which the disease germ leaves the body. In typhoid fever the urine and feces are likely to contain the specific germ; in consumption, the sputum.

The discharges from bowel and bladder should be received into a vessel containing a 5 per cent. solution of carbolic acid or fresh-prepared milk of lime made by adding one part of dry, freshly slaked lime to four parts of water. Slaked lime is prepared from quicklime by adding approximately one part of water to two parts of quicklime. The volume of the carbolic acid or milk of lime solution should be, at best, twice as great as the volume of the discharge. Thorough mixing and stirring are advisable, and solid masses of feces should be broken up. The mixture should stand for one hour before being thrown into the water-closet. The same treatment should be used for vomited material. The sputum from consumptive and pneumonic patients should be received in cups which contain a 5 per cent. carbolic acid solution or milk of lime. Paper cups may be used and burned with their contents. In the large number of other diseases in which the discharges from mouth and nose are infectious, care must be taken to prevent the dissemination of germs by sneezing or coughing, and patients should be instructed on this point. Soiled handkerchiefs and cloths may be boiled after immersion in the carbolic acid solution for one hour. In general, bed-clothing, towels, napkins, and cotton underclothing may be treated in the same fashion. Blankets, woolen clothing, mattresses, etc., may be exposed to steam, hot air, or formaldehyd gas in one of the large forms of apparatus provided by boards of health, or if of slight value, burned.

Not only clothes, but all other articles coming into more or less direct contact with a patient or convalescent, and hence liable to be

contaminated with epithelial scales or discharges from the mouth, nose, bladder, or bowels, must be carefully disinfected. Dishes and table implements should be provided for the exclusive use of the patient throughout his illness. After using they should be kept in hot water at or near the boiling-point for fifteen to twenty minutes. Articles of food—milk, for example—brought into the sick-room and remaining unused should be destroyed. Toys and books used by the patient and not of great value should be burned. Valuable books may be disinfected in a special formaldehyd chamber. Unopened books in book-cases whose surfaces only have been exposed will be satisfactorily disinfected in the course of the ordinary formaldehyd room disinfection. Woodwork and wooden furniture may be thoroughly washed with corrosive sublimate, 1 : 1000. Upholstered furniture, rugs, and carpets, if allowed to remain in the room at all, are difficult to disinfect, and, especially if soiled with discharges, need to be treated in a special formaldehyd chamber. The hands of the patient and of nurses and attendants need particular attention. After a thorough cleansing with 2 per cent. solution of carbolic acid or a 1 : 1000 solution of mercuric chlorid they should be washed with soap and water. This should always be done before eating. Parts of the body that become soiled with discharges should be immediately cleansed in the same way.

Room disinfection may be carried out with formaldehyd gas generated in one of the ways already specified. The room must be tightly sealed, a temperature of at least 10° C. (50° F.) must be maintained, and the atmosphere must contain at least 75 per cent. of moisture. Sleeping-cars, ambulances, and the like may be disinfected by the same means.

For surgical operations thoroughly cleansed instruments may be sterilized by boiling for one minute in a 1 per cent. soda solution. Rubber gloves for the hands of the operator and assistants are now generally used in surgical operations and may be sterilized in the same way. The skin of the patient may be first washed scrupulously with alcohol and then with a 1 : 1000 solution of mercuric chlorid. Painting the skin with tincture of iodin is still simpler and is highly effective. Bandages, towels, gauze, surgeons' gowns and caps are usually sterilized by heat. Syringes may be sterilized by boiling for fifteen to twenty minutes, preferably in water to which 2 per cent. of soda is added.

CHAPTER V

THE EFFECTS PRODUCED BY BACTERIAL GROWTH

In the preceding chapter it has been shown that bacteria may be greatly modified in all their functional activities by the character of their surroundings. They are not, however, mere passive victims of their environment. The influence exerted by the higher forms of life upon surrounding objects is often impressive, and bacteria also can react upon their environment in a direct and sometimes surprising fashion. Relatively slight physical and chemical changes in bacterial surroundings may give rise to a remarkable and profound disturbance of the surroundings themselves. The rapid invasion of the animal body by bacteria and the resultant putrefactive change which takes place soon after death is a familiar instance. The infection of the body of the fowl by the anthrax bacillus, which has no effect upon the normal animal, but gains a foothold and effects injury when the temperature of the fowl is lowered only a few degrees below the normal, affords another example. The variations in the nature of the bacterial products due to slight changes in nutrient media offer innumerable illustrations of the reactions of bacteria upon their surroundings in response to relatively insignificant environmental changes.

Physical Effects.—Both heat and light may be generated by bacterial growth. As would be expected from chemical considerations, the temperature of organic substances undergoing bacterial decomposition is frequently raised high above that of the surroundings. The heating of manure piles or of damp hay is often classed as a bacterial phenomenon. It is even thought that some cases of "spontaneous combustion" should be attributed to the agency of the thermogenic bacteria, and that although the train of events leading to the actual bursting into flame is not fully understood, bacteria play a part in the initial stages of the process. Boekhout and de Vries[1] maintain that the self-heating of the hay is of a

[1] Boekhout and de Vries: Centralbl. f. Bakt., II, 1904, 12, p. 675; 1908, 21, p. 398.

purely chemical nature. Miehe,[1] in an exhaustive monograph on the subject, adduces strong evidence of the thermogenic power of certain micro-organisms. The latest observations, however, support the view that the heating process is due to chemical reaction unaided by bacterial activity.

The phosphorescence sometimes observed upon decaying fish and meat is due to the growth of light-producing bacteria. Sodium chlorid and magnesium chlorid favor the growth of these phosphorescent bacteria, and one or the other of these salts is essential to the production of light. Aërobic conditions are absolutely necessary for photogenesis. The photogenic bacteria are found most commonly, though by no means exclusively, in sea-water and upon the bodies of marine animals. As many as 28 different species have been enumerated. The light generated by active cultures of these organisms is considerable; photographs of cultures have been taken by their own illumination.[2] It is supposed that a substance in the living cell—photogen—is responsible for the light phenomena. Photogen, like zymase, is closely bound to the cell protoplasm; but unlike zymase, photogen has not yet been freed by pressure and filtration from the living cell.

The dependence of bio-luminescence upon oxygen is beautifully illustrated by the experiment of Beijerinck.[3] He added a ground, chlorophyl-bearing leaf to a suspension of luminous bacteria in sea-water. After the dissolved oxygen had been used up and the luminescence had disappeared, the solution again became luminous if a burning match or other source of light was brought near the tube. The chloroplasts decomposed the dissolved CO_2 and liberated the oxygen. Harvey and Morrison[4] have measured the concentration of oxygen which is but just sufficient for the development of perceptible luminescence by bacteria isolated from fish. The value proves to be exceedingly low, being but 0.005 mm. Hg. partial pressure of oxygen, equivalent to one part of oxygen (by weight) dissolved in 3,700,000,000 c.c. sea-water.

Chemical Products.—From a physiologic standpoint the substances produced by bacterial life and activity may be divided

[1] Miehe: "Die Selbsterhitzung des Heues," Jena, 1907, p. 127.

[2] See especially Molisch, H.: "Leuchtende Pflanzen," Jena, 1904, pp. 121–151.

[3] Beijerinck: Kon. akad. van wetensch. te Amst., 1902, 4, p. 45.

[4] Harvey and Morrison: Jour. Gen. Physiol., 1923, 6, p. 13.

conveniently into four classes: (1) The *secretions*, or those substances which subserve some purposeful end in the cell-economy; these may be retained inside the cell or they may pass out into the surrounding medium. (2) The *excretions*, or those substances that are ejected because useless to the organism: the ashes of cell-metabolism. (3) The *disintegration products*, or those bodies that are produced by the breaking-down of food substances; their nature is determined partly by the chemical structure of the nutrient, partly by the specific bacteria concerned in the disintegration; some of the most conspicuous, if not the most important, of bacterial products belong to this class; enzyme action is largely responsible for their existence. (4) The true *cell-substance*. Under this head may properly be included the protoplasm itself, substances in the early stages of assimilation that are on the way to become protoplasm, and substances that are being broken down but have not reached the stage where they are cast out of the cell.

Even on the basis of such a classification it is not always easy to assign to any given bacterial product its proper significance. Enzymes can readily be placed in the class of secretions, but the physiologic meaning of bacterial pigments, for instance, is obscure. It is variously held that the pigments are disintegration products, that they are excretions, or even that they are secretions.

The Production of Pigment.—Most bacterial cells do not contain pigment, and a mass of bacteria—an agar culture of B. typhosus, for example—has to the naked eye a muddy gray tint. Some kinds of bacteria, however, in the course of their growth, give rise to colored substances, often of brilliant hue. Some pigments occur in solution; others in the form of granules outside of the cell in the nutrient substratum. Practically all colors of the spectrum are represented: violet, indigo, blue (B. violaceus, B. janthinus, B. cyanogenes, B. pyocyaneus); green (B. fluorescens); yellow (Staphylococcus aureus, Sarcina lutea); orange (Sarcina aurantiaca), and red (B. prodigiosus). The last-named, the socalled "miracle" micro-organism, is sometimes the cause of the sudden appearance of blood-red spots on polenta, rice, fish and other food substances.[1]

[1] The name Serratia was given to this organism by Bizio in 1823 and has been revived in some recent systems of classification. Harrison (Trans. Roy. Soc. Canada, 1924, 18) has collected an interesting record of epidemics of "bloody bread," etc.

Great variation in the amount and character of the pigment produced by one and the same species may occur; cultivation on the ordinary media often occasions the temporary or permanent loss of chromogenic power (B. violaceus), and growth at an unusual temperature may have a similar effect (B. prodigiosus at 37°). Some species that are not usually regarded as chromogenic may give rise to colored sports (*e.g.*, B. diphtheriæ, Hill). As a rule, oxygen is indispensable to pigment production, and most chromogenic species yield no trace of pigment when grown under anaërobic conditions. Spirillum rubrum, however, which grows well in the presence of oxygen, is said to form its red pigment only in oxygen-free media. In the case of some chromogens the presence of certain chemical compounds or elements in the nutrient media is essential to, or greatly favors, pigment production. Thus phosphates and sulfates have been found necessary for the production of pyocyanin by B. pyocyaneus, and sodium tartrate has been shown to favor the production of pigment by B. prodigiosus. Carbohydrate media (potato, rice, and wheat starch) often lead to a particularly brilliant chromogenesis. Antiseptics may check or altogether inhibit pigment production. The bacterial pigments are chemically of diverse nature. Many of the red and yellow pigments are insoluble in water, but soluble in alcohol, ether, and chloroform. Others, like the fluorescent pigment, are soluble in water, but not in ether or strong alcohol. Some, and possibly the majority, are chemically related to the lipochromes, a group of fatty pigments widely distributed throughout the plant and animal kingdom. The reactions of others suggest an affinity to certain anilin dyes, such as fuchsin.

The relation of the bacterial pigments to the physiology of the individual cell is a debated point. It is held by some that the pigments are mere by-products that have no particular meaning for the organisms forming them, and that their formation is an incidental, and not an essential, feature of the cell-metabolism. As regards the majority of bacterial pigments, there is much to support this position. It is maintained by others, however, that at least some pigments enter into a loose combination with oxygen, analogous to the union effected by hemoglobin, and that under certain circumstances oxygen may be liberated. It has been suggested, further, that the pigments serve to protect the bacteria producing

them from the action of light, but experimental evidence is against this view.

Enzymes[1] and Fermentation Products.—It is well established that many of the chemical effects wrought by bacteria, as by other living cells, are due, not to the direct action of the protoplasm, but to the intervention of soluble ferments or enzymes. Probably the majority of the disintegrative processes in which bacteria are concerned are carried on by means of these powerful protoplasmic auxiliaries. In many cases the enzymes diffuse out from the cell and exert their effect upon the ambient substances, as do, for example, the gelatinases or gelatin-liquefying enzymes; in others the enzyme action occurs within the cell and the products pass out. The zymase or alcohol-producing enzyme of the yeast-cell apparently does not diffuse out, but acts upon sugar within the cell, the resulting alcohol and carbon dioxid being ejected. The difference between enzyme action within and without the cell would not seem to be a fundamental one.

It is in accord with the great adaptability shown by bacteria in their utilization of various food-substances that the list of enzymes known to be secreted by different species is a long one. A comprehensive review of the enzymes produced by micro-organisms, with full bibliography, has been given by Waksman.[2] Probably all classes of enzymes are represented among bacterial products, although in some cases where there is reason to suspect enzyme action no enzyme has yet been demonstrated. Some of the changes in nutrient media that are most relied upon as differentiation marks are effects produced by enzymes, such as the liquefaction of gelatin, the precipitation of casein, the dissolving of casein, and the inversion of sugar. A single form of micro-organism may secrete more than one kind of enzyme, and some species are known to give rise to a large number. Different kinds of enzymes are formed under the influence of different conditions of life, the nature of the nutrient substratum being especially determinative. The presence of a particular carbohydrate, for example, may stimulate a bacterial species to produce a hydrolytic enzyme, which under other circumstances is not found among the products of that species.

[1] See Fuhrmann: "Vorlesungen über Bakterienenzyme," Jena, 1907; Waksman and Davison: "Enzymes," Baltimore, 1926.

[2] Waksman: Abstr. Bact., 1922, 6, p. 265.

The term fermentation has been used, and to some extent is still used, to express various conceptions. A sharp distinction between changes produced by the living cell and changes produced by enzyme action is no longer tenable, since many of the effects once ascribed to "living ferments" have been shown to be directly attributable to cell-secreted enzymes. The discovery of the alcohol-producing enzyme, zymase, has removed almost the last excuse for limiting the term fermentation to direct protoplasmic interference. More recently the nature of the substances acted upon has been made the basis of distinction. The tendency at present is to limit the term fermentation to the disintegration of carbohydrate substances, and there are some who would go so far as to consider as true fermentations only those carbohydrate decompositions in which gas is produced, a virtual reversion to the old etymologic signification (*fervere*, to boil). In ordinary descriptions it is customary to state that B. typhosus, for example, does not ferment lactose, that Staphylococcus aureus ferments lactose with production of acid, and that B. coli ferments lactose with production of acid and gas. Proteolytic action is not usually denoted as fermentation, though logically the putrefaction of protein substances by bacterial agency falls in the same category with the decomposition of sugar by the action of the yeast-cell.

The large group of disintegrative products can only be briefly touched upon here. A considerable portion of this book is devoted to the description of the various activities and products of important microbes, and the reader will soon become aware that a notable share of the interest that attaches to certain species is due to the nature of the chemical changes wrought by them in the surrounding food-substances.

The "Iron Bacteria."—Among the trichomycetes (p. 528) or filamentous bacteria are found some varieties especially characterized by deposits of iron oxid in the sheath or sometimes in the protoplasm. The best known of these organisms are the widely spread Crenothrix polyspora, Leptothrix ochracea, and Spirophyllum ferrugineum, which sometimes grow in the conduits of certain public water supplies, where they form unpleasant-looking, brownish, flocculent masses, often leading to complete stoppage of the pipes. The frequent appearance of detached portions of the growth in tap-water gives rise to consternation among the water consumers,

as in the famous "water calamities" in Berlin, Lille, Rotterdam, and other places. There is no evidence that such organisms are directly harmful.

Winogradsky, explaining the presence of iron in the sheath by the oxidation of iron in the cell protoplasm of Crenothrix, asserted that the presence of some iron salt was indispensable to the growth of the micro-organism, and attached great significance to the physiologic activity of the iron bacteria in causing the deposit of iron from solution, and the consequent formation of great beds of mineral iron in the earth's crust. Molisch,[1] however, concluded from his investigations of bog-iron ore from various sources that these micro-organisms were by no means universally concerned in the deposition of iron ore on a large scale, but that under certain natural conditions well-known physicochemical agencies might play an important part in the process. The latter author has also asserted from experimental observations on Chlamydothrix ochracea that iron is simply deposited by external chemical action on the sheath, and no vital process is at all concerned therein, and further, that this organism can live in an iron-free medium, and is capable of storing up manganese as well as iron. Lieske,[2] on the other hand, has found that in the case of Spirophyllum ferrugineum the iron is built up chemosynthetically by the protoplasm of the plant from ferrous carbonate, which constitutes a real and necessary source of energy. He was unable to grow the organism in an iron-free medium or induce it to utilize the salts of other metals.

A valuable critical study of iron-depositing bacteria and their geologic relations has been made by Harder,[3] who concludes that there are three principal groups of iron-depositing bacteria: (1) Those, of which Spirophyllum ferrugineum is a type, that precipitate ferric hydroxid from solutions of ferrous bicarbonate and use the carbon dioxid liberated and the energy produced during oxidation for their life processes; (2) those, represented by Leptothrix ochracea, that do not require ferrous bicarbonate for their life processes, but that cause the deposition of ferric hydroxid when either inorganic or organic iron salts are present; (3) those, probably including a number of the lower or ordinary water and soil bacteria,

[1] Molisch: "Die Eisenbakterien," Jena, 1910.

[2] Lieske: Jahrb. f. wiss. Bot., 1911, 49, p. 91.

[3] Harder: U. S. Geological Survey, Professional Paper 113, Washington, 1919.

that attack organic iron salts, using the organic acid radicle as food and precipitating ferric hydroxid or basic ferric salts, which are gradually changed to ferric hydroxid. These organisms cannot utilize inorganic iron salts.

There seems little doubt that iron-depositing bacteria have played an important part in the formation of iron-ore deposits, but the relative share of chemical and biologic processes in an individual case can be determined only by a thorough study of conditions at the time of deposition, especially as regards sedimentation, climate, depth of water, nature of material in solution, and other factors.

The "Sulfur Bacteria."—Sulfur, like nitrogen, is an essential constituent of living matter. When organic matter is decomposed by bacteria, sulfuretted hydrogen (H_2S) is one of the usual disintegration products. If anaërobic conditions prevail or if the medium is rich in sulfur compounds, as, for instance, is the case with the yolk of eggs, the odor of H_2S is usually plainly perceptible, and has come to be recognized as one of the most familiar signs of decomposition. The sulfuretted hydrogen may arise either from the splitting off of H_2S groups already present in the molecule or from the reducing action of the bacteria upon the protein substance. Sulfuretted hydrogen may also be formed by the reduction of inorganic sulfur compounds, such as sulfates, sulfites, and thiosulfates. It has been shown by Myers[1] that oxidized sulfur (taurin) is not readily attacked by bacteria, while partially reduced sulfur (cystin) is completely reduced to hydrogen sulfid. Many different kinds of bacteria, including most of the common laboratory organisms, are able to generate sulfuretted hydrogen from protein bodies. The power of reducing sulfates, however, seems to be a quality less widely shared and is not possessed, for example, by such bacteria as Bacillus coli, which reduces nitrates vigorously. Beijerinck was the first to isolate a special micro-organism, Spirillum desulfuricans, which he regarded as the peculiar organism of sulfur reduction. At least two other forms have since been described. Sulfate-reducing anaërobic bacteria have been found in water from producing oil wells over 3000 feet in depth in Illinois and California.[2] It has been recognized for some time that waters associated with petroleum in

[1] J. T. Myers: Jour. Bact., 1920, 5, p. 231.

[2] Bastin, Greer, et al: Bull. Amer. Assn. Petroleum Geologists, 1926, 10, p. 1270.

oil pools are so low in sulfates as to indicate that a natural reduction of sulfates has been in progress, but until recently this was attributed by geologists to the petroleum.

The direct physiologic opposite of these reducing bacteria are the *sulfur bacteria* proper, which are able to exert a strongly oxidizing action upon sulfuretted hydrogen. These organisms are found in the water of sulfur springs, in sewage-laden streams, in swamps where masses of vegetable matter are slowly decomposing, and, in fact, wherever an abundance of sulfuretted hydrogen is being liberated. Two genera are especially recognized: *Beggiatoa* and *Thiothrix*. The former is a long cylindric filament possessed of the power of active movement and showing a close morphologic resemblance to the blue-green alga Oscillaria. Thiothrix is differentiated from Beggiatoa by its lack of motility, by the possession of a sheath, and by the formation of so-called conidia or spores. A group of non-filamentous, colorless sulfur bacteria also exists (Thiophysa, etc.), but has thus far been little investigated. The so-called red or purple sulfur bacteria are peculiarly interesting. These organisms were observed in 1826 by Ehrenberg, and in recent years have been studied especially by Winogradsky, who has placed them in a family by themselves, called the Rhodobacteriaceæ. They are found especially in places where a vigorous reduction of sulfates is taking place and where, consequently, sulfuretted hydrogen is present in great abundance. The pigment which imparts the characteristic color to these organisms gives some of the reactions of the lipochromes, but little is definitely known about its composition. Unlike the unpigmented sulfur bacteria, which are completely indifferent to light, the purple bacteria gather by preference on the light side of an aquarium. The view of Engelmann, however, that the pigment is similar to chlorophyl and that the purple bacteria, like the green plants, give off oxygen in the sunshine, has not been confirmed.

The physiology of the group of sulfur bacteria is unlike that of any other living organisms, and they deserve to be set apart, as Winogradsky has proposed, as an independent physiologic group. The true sulfur bacteria all contain in their protoplasm highly refractive inclusions which have been found to be amorphous sulfur. The presence of sulfur in the cell is undoubtedly connected with the fact that the organisms are found abundantly only in waters con-

taining sulfuretted hydrogen. The discovery of the full physiologic significance of these findings has been largely the work of Winogradsky. By the oxidation of sulfuretted hydrogen to sulfuric acid, which is, of course, at once neutralized by the carbonates present, the sulfur bacteria obtain the energy necessary for their development. Sulfuretted hydrogen, in a word, is their principal food. According to Winogradsky, the single Beggiatoa threads use in a day two to four times their own weight of H_2S. The sulfur in the cell protoplasm is to be looked upon as an intermediate stage in the oxidation process. The course of the reaction may be indicated by the following equations:

$$(1)\ 2H_2S + O_2 = 2H_2O + S_2.$$
$$(2)\ S_2 + 3O_2 + 2H_2O = 2H_2SO_4.$$

That sulfuretted hydrogen is indispensable for the continued activity of these organisms and is for them the sole available source of energy is inferred from the fact that if it is not accessible the store of sulfur in the cells quickly disappears (in twenty-four to forty-eight hours) and the bacteria apparently then die from starvation. It seems probable that the sulfur bacteria require no organic substances for their development, but that, like the nitrite bacteria (p. 656), they can subsist on a purely mineral diet. For these organisms, therefore, sulfur in its combination with hydrogen seems to have the same physiologic value that carbon in its hydrogen compounds has for most other bacteria.

The Production of Acid and Alkali.—The production of acid and alkali by bacteria is one manifestation of enzyme action. The occurrence of acid production is so commonly used as a means of special differentiation that it is convenient to consider this topic separately. In general it may be said that an acid reaction is caused by the fermentation of some sugar, alcohol, or similar body present in the nutrient medium. The muscle-sugar in nutrient broth made from fresh meat, the lactose in milk and whey, and other sugars naturally present in, or artificially introduced into, various culture media, are usually responsible for the occurrence of an acid reaction in the medium as a sequence to bacterial growth. In a word, the production of acid by a given species is due to its ability to break up some chemical substances in such a way that hydrogen ions are liberated. Thus the colon bacillus ferments lactose and saccharose, and in consequence provokes an acid fermentation in media contain-

ing these carbohydrates, while the typhoid bacillus is unable to effect this change. In a peptone-mannite solution, on the other hand, the typhoid bacillus produces acid in twenty hours at 37° while B. coli leaves the original reaction unaltered.[1] The power of acid production, or, more narrowly, the ability to ferment certain sugars, is one of the more constant physiologic characteristics of bacteria, and has been used advantageously to distinguish closely allied organisms, notably in the groups of paratyphoid and dysentery bacilli.

The acid reaction produced by a given organism in a medium containing a utilizable carbohydrate eventually reaches a point where further growth of the organism is inhibited. This final reaction is termed the limiting hydrogen-ion concentration for that organism. Ayers,[2] Avery and Cullen,[3] and others have shown that virulent hemolytic streptococci of human origin may be differentiated from hemolytic streptococci of bovine type by differences in their final hydrogen-ion concentrations.

Carbohydrates are not the only substances the breaking down of which is accompanied by an acid reaction. The liquefaction of gelatin by bacteria gives rise to a noteworthy increase in the acidity of the medium.

Alkali production is sometimes declared to be more intimately bound up with the constructive (anabolic) side of bacterial metabolism than with its destructive aspect, but the real difference between alkali and acid production lies in the nature of the substances attacked. In sugar-free nutrient broth the majority of bacterial species produce an alkaline reaction due to the formation of ammonia. This is more marked with some kinds than with others. B. pyocyaneus, B. suipestifer, and B. diphtheriæ are among the especially vigorous producers of alkaline substances. The alkalinity of a culture undoubtedly depends in most cases upon the fact that the food-substances are disintegrated by the bacterial cells or their enzymes in such a way as to yield bodies that give rise to free hydroxyl ions. Protein substances, as a rule, break up in this way, while carbohydrates, when they are attacked at all, give rise to acids. Under suitable conditions bacteria may produce acid and alkaline fermentations simultaneously,[4] as by forming acids from carbohydrates and alkaline carbonates from the organic acids.

[1] Capaldi and Proskauer: Ztschr. f. Hyg., 1896, 23, p. 452.
[2] S. H. Ayers: Jour. Infect. Dis., 1918, 23, p. 290.
[3] O. T. Avery and G. E. Cullen: Jour. Exp. Med., 1919, 29, p. 215.
[4] Ayers and Rupp: Jour. Infect. Dis., 1918, 23, p. 188.

Putrefactive Products.—The decomposition of nitrogenous compounds constitutes a striking feature of bacterial activity, and one that has always claimed attention. One reason for the conspicuous character of protein decomposition, apart from the profound modifications that are observed to occur in the dead animal or plant body, is the frequent production of malodorous compounds like mercaptan and skatol, which obtrusively betray the neighborhood of decaying nitrogenous substances. The gases arising from the disintegration of proteids are numerous and varied. Ammonia, carbon dioxid, hydrogen, marsh-gas, sulfuretted hydrogen, and nitrogen are among the more common gases generated by bacterial action. A variety of volatile compounds, amids, peptones, and aromatic bodies, are also formed in the course of the complicated processes of putrefaction. The substances generated under anaërobic conditions differ materially from those formed in the presence of oxygen, it being well known, for example, that anaërobic decompositions are peculiarly apt to be accompanied by the evolution of offensive gases. Many writers believe[1] that only obligate anaërobes, and not all of those, are able to bring about putrefactive changes in native proteins. In general, it may be said that the action of bacteria upon proteins is very similar to the action of tryptic digestion, and results first in the formation of albumoses and peptones, which are then broken up into amino-acids. The amino-acids themselves are excellent nutrients for bacteria, as shown by Czapek,[2] and are split both by the elimination of ammonia and by the splitting off of carbon dioxid. Free fatty acids, aromatic acids, and certain ptomains, cadaverin and putrescin, are among the further products of decomposition.

Indol is a substance which has assumed importance as an aid to the differentiation of bacterial species. It is one of the final products of the decomposition of albuminous bodies, belongs to the aromatic series (C_8H_7N), and is characterized by a peculiar odor. It gives a red color reaction when strong nitric or sulfuric acid and a 0.01 per cent. solution of sodium nitrite are added drop by drop to a solution containing it, as, for instance, a peptone culture of an indol-producing organism. It also produces a cherry-red color when an acid solution of dimethylamidobenzaldehyd is added

[1] See Rettger: Jour. Biol. Chem., 1908, 4, p. 45.
[2] Czapek: Hofmeister's Beitr., 1902, 1, p. 538.

to an indol solution; this is a more delicate and accurate test than the former.[1] (See p. 37.)

The Relation of Bacteria to Food Assimilation by Higher Forms of Life.—The question whether the bacteria usually so abundantly present in the alimentary tract of man and the higher animals play a useful or a harmful part has been the theme of considerable speculation and some experimentation. Nuttall and Thierfelder, who were among the first experimenters in this field, succeeded in raising aseptically guinea-pigs that had been removed by Cesarean section from the body of the mother. The young animals, which were fed on sterile milk, lived for as long as ten days after birth and their weight increased as much as 28 grams. From such facts these investigators concluded that the presence of bacteria in the digestive tract is not indispensable for the life of the guinea-pig; by analogy they infer that the same would be true for the other higher animals and for man. Schottelius[2] vigorously combats this view on the basis of his own experience in raising sterile chicks. In the conduct of these experiments, the egg-shells were carefully washed with corrosive sublimate, which was then completely removed by chemical neutralization; this procedure is not injurious, since control eggs yield perfectly normal chicks. Throughout Schottelius' experiments complete bacterial tests of the food, air, water, and dejections were made at the beginning and the close of each series. The bacteria-free chicks were apparently hungrier than the others and ate more greedily, but in spite of this fact always lost weight and usually died before reaching the thirtieth day. When some of the sterile chicks were separated from the others and given food contaminated with fresh fowl droppings, they always throve better than the control chicks and usually gained weight and grew to maturity. When a pure culture of a variety of Bacillus coli was used to inoculate the food equally good results were obtained. A mixture of air-cocci with the food was not so favorable. From these results Schottelius concludes that the bacteria normally present in the digestive tract have a marked beneficial influence and are in reality necessary to the life of the higher animals. Schottelius further maintains that the increase in weight of the guinea-pigs in Nuttall and Thierfelder's experiments was due not to a genuine tissue construction, but to the presence of coagulated and undigested milk in the alimentary canal.

[1] Böhme: Centralbl. f. Bakt., 1905, Abt. I, Orig., 40, p. 129.

[2] Schottelius: Arch. f. Hyg., 1902, 42, p. 48, and 1908, 67, p. 177.

Cohendy,[1] on the other hand, has reached conclusions quite at variance with those of Schottelius. By the use of more elaborate and, it is thought, more suitable methods of raising young chicks in the laboratory, Cohendy succeeded in bringing about "life without microbes." This was achieved in a vertebrate animal provided ordinarily with a rich intestinal microbic flora. The chicks raised aseptically were at least as robust as those raised under ordinary conditions. The discrepancies between Cohendy's observations and the work of Schottelius still need elucidation.

Ptomains and Toxins.—For evident reasons a high degree of interest attaches to the poisonous products of bacteria. As might be anticipated, these products differ in respect to their origin and physiologic significance.

It may happen during the course of the decomposition of organic substances that toxic bodies are produced simply as a consequence of the mode of disintegration of the protein molecule. Among such poisonous products of decomposition, for example, are a few substances belonging to the group of alkaloid-like bodies known as *ptomains*, basic compounds characterized by a more or less definite chemical composition. Investigators once supposed that in the decomposition of meat, fish, cheese, and the like, poisonous ptomains are formed in such quantities that the ingestion of partly decayed food can cause acute poisoning. It is possible that cases of "ptomain poisoning" in man due to ingestion of ptomains or to their formation within the intestine sometimes occur, but there is no doubt that such cases, if they occur at all, are very rare. Many of the epidemics of "meat poisoning," etc., are now known to be due to infection with specific micro-organisms, rather than to the action of a formed poison. In the case of botulism (p. 418), which is caused by a bacterial poison formed outside the body, the poison is a true bacterial toxin very different in character from the ptomains. It still remains to be proved that the ptomains play any really important part either in isolated cases or in outbreaks of food-poisoning, or in the so-called gastro-intestinal auto-intoxications.

The nature of the poisons produced by bacteria in the living body has been much debated. There is reason to think that they are not cleavage products, but are closely related to the life of the bacterial cell. At one time the specific bacterial poisons were

[1] Cohendy: Ann. de l'Inst. Past., 1912, 26, p. 106.

believed to belong to the class of ptomains just referred to, but this view was abandoned when it was shown that the ptomains apparently generated by certain pathogenic bacteria were not able to reproduce the appropriate symptom-complex of any disease, and did not correspond in other respects with the toxicologic requirements. There is, furthermore, some reason to look upon at least a portion of the ptomains isolated from decomposing substances and from bacterial cultures as secondary products due to too drastic methods of chemical manipulation, and not as the primary products of bacterial activity.

The opinion that the specific bacterial toxins belong to the class of proteins or albuminous substances has found strong support. It has, at all events, not been found possible by extensive chemical study to obtain a protein-free substance which has the characteristic properties of diphtheria or tetanus toxin. Since the proteins are the only chemical substances proved to be capable of giving rise to antibody production, and since the bacterial toxins produce specific antibodies, this has been regarded as important evidence in favor of their protein nature.

The true bacterial *toxins*, in the modern acceptation of the term, are specific poisonous metabolic products of bacteria. They are of completely unknown chemical structure, are probably colloidal, are extraordinarily labile, and display great sensitiveness toward heat. They are soluble and pass out from the bacterial cell into the culture-medium, hence they are termed soluble toxins or exotoxins. In many respects they are closely analogous to the enzymes. One of the most characteristic qualities of the true toxin is its ability to evoke the formation of a specific antibody, an antitoxin, when injected into the body of a suitable animal species. The potency of the bacterial toxins is extraordinary, and far surpasses that of any other known poison:

Minimal fatal dose of atropin for adult man.....130 mg.
" " " " strychnin for adult man.....30 to 40 mg.
" " " " cobra venom for adult man.....4.375 mg.
" " " " tetanus toxin for adult man.....less than 0.23 mg.

Some bacteria, as the tetanus bacillus, are able to produce their specific toxin in the animal body; others, as Cl. botulinum (p. 420), form their toxin, so far as known, only in organic substances outside of the living body. A characteristic feature of the action of toxins,

although one not fully understood, is the period of incubation that elapses after the toxin is introduced into the body before the symptoms become manifest. Tetanus toxin injected into a horse may not cause symptoms for as long as four or five days.

The constitution of the toxin molecule has been the subject of much study, especially by Ehrlich and his coadjutors. Elaborate experiments have shown that the toxin is a complex substance. To take a specific illustration, it is found that broth in which the diphtheria bacillus has grown loses, on standing, a certain part of its toxicity, but retains undiminished its affinity for diphtheria antitoxin. In other words, there is no constant relation between the toxic strength of the broth and the amount of the broth that is neutralized by a given quantity of diphtheria antitoxin. This is held to indicate that the toxin is not a simple chemical unit, but is composed of two portions, distinguished by their different stability. Exposure to light and air destroys the toxic portion of the toxin-complex, but leaves the combining portion intact. Hence a toxin has been supposed to consist of two portions: a combining or *haptophore* atom-group, which is able to unite with the corresponding antitoxin, and a specific *toxophore* atom-group, to which the poisonous action is due. Those modifications of the toxin-complex from which the toxophore portion has more or less completely disappeared, while the haptophore group persists, have been designated as *toxoids* or *antitoxins*.

The name *toxon* has been given to a poisonous product of bacterial growth possessing the same haptophore group as the toxin, but of far less avidity. The toxophore group of the diphtheria toxon is conceived as different from the toxophore group of the diphtheria toxin, in that it is incapable of producing acute effects, cutaneous necrosis, and death; but, on the other hand, it is responsible for the characteristic late diphtheria paralysis. Analysis of the phenomena attending the neutralization of toxins and antitoxins of various strengths led Ehrlich to a belief in the existence in toxic broth of a variety of toxic bodies possessing varying degrees of avidity and toxicity. These are the so-called prototoxins, deuterotoxins, prototoxoids, etc. It has been urged against this view by Arrhenius and Madsen,[1] on physicochemical grounds, that the behavior of mixtures of toxin and antitoxin can be explained by reference to

[1] Arrhenius and Madsen: Zeit. f. physik. Chemie, 1903, 44, p. 7.

the law of mass-action enunciated by Guldberg and Waage. The law of mass-action declares that chemical reaction goes on at a velocity proportionate to the concentration of the reacting molecules. On the latter hypothesis it would be superfluous to assume the existence of different components, and sufficient to regard the toxin as a single uniform substance possessed of a weak affinity for its antitoxin. Further researches have shown that it is not possible to bring the reaction of toxin and antitoxin within the law of mass-action, and that the relations of these two bodies to one another are much more complicated than would be expected if they were simple crystalloidal substances. On the other hand, certain striking analogies have been shown to exist between the behavior of toxins and the behavior of colloidal substances, and it seems possible that the phenomena seen in mixtures of toxin and antitoxin may be due to a reaction between two colloids. Grassberger and Schattenfroh[1] have found that the poison of the bacillus of symptomatic anthrax is free from toxoids and toxons, but that, nevertheless, toxin and antitoxin mixtures unite in variable proportions.

All of the foregoing statements relate to the so-called *exotoxins* or *extracellular toxins*, that is, to those toxins of which tetanus toxin and diphtheria toxin are the types, which diffuse through the bacterial cell wall during life and are found in the fluid culture-media in which the bacteria are grown.

Relatively few pathogenic bacteria have been shown to produce exotoxins. The best known in addition to those produced by the diphtheria bacillus and the tetanus bacillus are the toxin of Bacillus botulinus and that of the bacillus of symptomatic anthrax. Under certain conditions soluble toxins have been found by some observers in cultures of the cholera vibrio, the dysentery bacillus, the typhoid and paratyphoid bacilli, B. pyocyaneus, and a few other organisms. But in none of these latter cases have the nature and pathogenic importance of these soluble toxins been demonstrated as they have for the four exotoxins first named.

The existence of another group of bacterial toxins, the so-called *endotoxins*, was indicated by the work of Pfeiffer (1892), who observed that guinea-pigs that had been immunized to the cholera vibrio nevertheless soon died if a too large dose of cholera vibrios was

[1] Grassberger and Schattenfroh: "Ueber die Beziehungen von Toxin und Antitoxin," Leipzig u. Wien, 1904.

injected. Cultures from the organs were sterile, showing that the infection was overcome. The death of such animals, in spite of the failure of the cholera vibrios to multiply in the body, was attributed to a poison present in the bacterial cells and liberated only by disintegration after cellular death. Endotoxins may be obtained by certain methods of extraction or by autolysis, but they differ strikingly from the exotoxins in not possessing specificity, and hence in being unable to give rise to antitoxins. It seems probable that the endotoxins are not definite preformed constituents of the bacterial cell, but that they are simply the poisonous products of disintegration of bacterial protein. Vaughan[1] has shown that a great variety of proteins, bacterial and otherwise, yield, upon cleavage with alkalinized alcohol, toxic split bodies which possess many of the properties of endotoxins. When these protein split products of non-bacterial origin are injected into animals they produce fever and other symptoms which usually accompany bacterial infection. The significance of endotoxins in the causation of diseases is quite obscure. It seems possible that pathogenic bacteria in which thus far only endotoxins have been demonstrated may produce true toxins under the conditions in which they grow in the animal body.

Substances possessing the closest resemblance to the true bacterial toxins occur in the seeds of some of the higher plants and in the secretions of certain animals. Among the best known of the vegetable toxins (phytotoxins) are ricin (from the castor-oil bean, *Ricinus communis*), abrin (from the jequirity bean, *Abrus precatorius*), and the similar substances, crotin and robin. These poisons, like the bacterial toxins, are exceedingly potent. It is estimated that 1 gram of ricin, properly diluted, is sufficient to cause the death of 1,500,000 guinea-pigs. Ricin not only exerts a strongly toxic effect upon the tissues at the seat of inoculation but also causes agglutination of the erythrocytes. After injection an incubation period is observed, as in case of the bacterial toxins. By use of the same methods as with the bacterial toxins, antiricin and the other corresponding antibodies may be produced, and the existence of haptophore and toxophore groups in these poisons is inferred on the same grounds. Specific toxic bodies are found in the blood and secretions of a number of animals (zoötoxins). Snake

[1] V. C. Vaughan: "Protein Split Products," Philadelphia, 1913.

venom and the poisons of scorpions and spiders, as well as an actively poisonous substance present in eel-blood, are more or less familiar examples. The chemical behavior and physiologic action of these poisons are strikingly similar to those of the true bacterial toxins. The snake venoms owe their power to a variety of active principles: (1) hemagglutinin, (2) hemorrhagin (present especially in rattlesnake venom), (3) hemolysin, and (4) neurotoxin. Antibodies have been successfully produced against these several toxic bodies. The action of the venins and antivenins is more complicated than that of the bacterial toxins.

The Bacteriophage Phenomenon.—It was observed by Twort in 1915 that transparent areas where there was no visible growth occurred in certain staphylococcus cultures, and that if one of these transparent areas was touched with a platinum loop and the loop then drawn across the surface of a young staphylococcus culture, the track of the loop would become clear and transparent within a few hours. This singular dissolving or lytic effect was manifested also when the material from the transparent areas was passed through a Berkefeld filter and added to an agar culture. d'Herelle a little later obtained a similar effect with filtered cultures of the dysentery bacillus and filtered stools from dysentery patients. The latter observer continued intensively his study of the phenomenon (often known as the d'Herelle phenomenon), and has published a comprehensive summary and discussion of his own experiments and those of others in this field.[1]

It was at first believed that the bacteriophages were specific, that each lytic filtrate would dissolve only the bacterial species from which it was derived, but this was disproved by later observations. The bacteriophages possess relatively high heat resistance, in some instances manifesting resistance to one-half hour's heating at 70° (Kuttner).[2] The effect of chemicals seems to need further study. The distinctive action of bacteriophage upon certain pathogenic bacteria, *e. g.*, the dysentery bacillus, has suggested its use as therapeutic agent; but while some observers have claimed success,

[1] d'Herelle: "The Bacteriophage and Its Behavior," Baltimore, 1926, pp. 629; for other observations and different interpretations of the phenomena see Bordet: Ann. de l'Inst. Pasteur, 1925, 39, p. 717; Hadley: Jour. Infect. Dis., 1927, 40, p. 1.

[2] Kuttner: Jour. Bact., 1923, 8, p. 49.

others have failed to notice the slightest benefit from the administration of antidysentery bacteriophages (Davison[1]).

Much discussion has centered around the nature of the bacteriophage, the two leading views being (1) that it is a living organism, an ultra-microscopic, filter-passing microbe, or (2) that it is an enzyme or enzyme-like body either produced by bacteria or by interaction between the microbe and the tissue cells of its host—"transmissible bacterial autolysis." The conclusion that the bacteriophage is a peculiar kind of micro-organism, a parasite on bacteria, has been upheld with great ingenuity and persistence by d'Herelle and his supporters, but the recent trend of opinion has been against this view. The relations of the bacteriophage phenomena to such bacteriolysis as is induced by alkali[2] and by certain other reagents (*i. e.*, bile acting on pneumococci, pyocyaneus cultures on anthrax, etc.) have not been studied extensively. They may be worthy of more attention than has been accorded them. Most of the experiments designed to identify the enzyme of bacteriolysis with the common digestive enzymes have been essentially unsuccessful.

For a time the view was generally accepted that, as maintained by d'Herelle, the bacteriophage is a particulate substance.[3] Bronfenbrenner,[4] however, has found that the active principle is capable of spreading from the focus radially, independently of the multiplication of bacteria and independently of gravity, and that the ratio of spread is conditioned by the density of the medium. The apparent particulate distribution may be due simply to the ready adsorption of the bacteriophage on the coarser colloidal particles of the medium. Again, Bronfenbrenner and Korb[5] have shown that an excess of 95 per cent. alcohol added to a filtrate containing bacteriophage causes in a few minutes a marked reduction in activity of the latter, but that the residual lytic activity is affected at a much slower rate; this appears more like a precipitation-adsorption phenomenon than a toxic action upon a living agent.

[1] Davison: Abstr. Bact., 1922, 6, p. 159.

[2] Kruse: Münch. med. Wchnschr., 1909, 56, p. 897.

[3] See *e. g.* Bronfenbrenner and Korb: Jour. Exper. Med., 1925, 42, p. 483; Marshall: Jour. Infect. Dis., 1925, 37, p. 126; McKinley and Holden: Jour. Infect. Dis., 1926, 39, p. 451.

[4] Bronfenbrenner: Jour. Exper. Med., 1927, 45, p. 887.

[5] Bronfenbrenner and Korb: Jour. Exp. Med., 1926, 43, p. 71.

A different type of objection to the theory that the bacteriophage is a living virus was found by Hadley in the parallelism between serologic and bacteriophagic response in B. typhosus and certain avian paratyphoid bacilli.[1] It was noted by this observer that "a bacteriophage evolved from an organism common to the intestinal tract of man, but absent from birds, may possess at once and in full strength . . . power to cause complete inhibition and lysis of organisms common to the intestinal tract of birds but . . . never found in the human intestine."

[1] Hadley: Proc. Soc. Exp. Biol. and Med., 1926, 23, p. 443.

CHAPTER VI

THE CLASSIFICATION OF BACTERIA

Bacteria are minute unicellular organisms generally classed as plants rather than as animals. It is well known that any strict dividing-line between animals and plants is an entirely arbitrary one, and that there is no general agreement among naturalists respecting what shall constitute a determinative plant or animal characteristic. As regards the simplest forms of life, the power of independent movement has been perhaps most widely advocated as a mark, admittedly arbitrary, of animal nature. Such a distinction would cause diatoms to be ranked as animals and some sporozoa as plants. Many bacteria, as is well known, are actively motile. It has already been pointed out that the cell-wall of bacteria, unlike that of plant cells in general, is not composed of cellulose. On the other hand, transition forms connect the typical bacteria with certain lower algæ and fungi universally recognized as true plants. Some of the filamentous blue-green algæ, of which the genus Oscillaria is an example, are closely related to undoubted bacteria, and this fact among others has disposed naturalists to place bacteria with the plants. As a matter of fact, little importance is now attached to such questions, since there is every reason to suppose that all living organisms are fundamentally alike and originated from a common state. It is among the lowest forms of life that divergences between animals and plants melt away, and organisms possessed of characteristics of both groups are found.

The chemistry of the tubercle bacillus has been studied more elaborately than that of any other bacterium and Long[1] concludes that from a chemical point of view the animal or plant nature of this organism is an open question. It is of great interest that the tubercle bacillus contains a typical nucleic acid of the animal type found in thymus, pancreas, sperm and spleen. It does not contain nucleic acid of the so-called plant type found in yeast and wheat. On the other hand its ability to synthesize such complex

[1] Long: Am. Rev. Tuberc., 1923, 8, p. 195.

substances as amino acids and purin bases with no other source of nitrogen than ammonia is distinctly a plant characteristic.

Bacteria, therefore, occupy an intermediate position between the vegetable and animal kingdoms, and it is largely considerations of convention and convenience that place them among the plants. Since they possess no chlorophyl, they must be regarded as fungi. From their method of vegetable multiplication by simple fission they are known as fission-fungi or *Schizomycetes* (Ger., *Spaltpilzen*).

Within the group of bacteria themselves classification is, for practical purposes, especially important.

In general, the classification of living organisms is based on morphologic characters which are, broadly speaking, less liable to sudden change and more apt to coincide with relationship by descent than with physiologic characters. Walking mammals (horses), flying mammals (bats), and swimming mammals (whales) are more fundamentally alike than flying mammals and flying insects. Both the wing of a butterfly and the wing of a bird serve the purpose of flight, but structurally the two kinds of wings are far apart, and the animals themselves belong to widely separated branches of the animal kingdom.

Bacteria, however, are of such minute size and the observable differences in structure are so slight that any classification grounded on morphologic characters meets with many difficulties. Microorganisms that resemble one another very closely in appearance may differ radically in respect to the chemical changes to which they give rise, or to the pathologic processes that they evoke. Since these pathologic processes and chemical changes are of great practical importance, bacteriologists have come to lay considerable stress upon physiologic qualities. There can be no valid objection to such a practice. The modes of nutrition of bacteria and the products of their growth must give a more correct insight into fundamental internal structure than can simple external form, especially for organisms that lie on the limit of invisibility.

An interesting attempt at classifying bacteria on a physiologic basis was made by Jensen.[1] On the basis of source of nutrition the following chief groups were distinguished:

(1) Bacteria which, like the green plants, need neither organic carbon nor organic nitrogen. These so-called autotrophic bacteria

[1] Jensen: Centralbl. f. Bakt., II, 1909, 22, p. 305.

can build up both carbohydrates and proteins out of carbon dioxid and inorganic salts.

(2) Bacteria which need organic carbon compounds, but can dispense with organic nitrogen. These bacteria are able to synthesize protein substances out of carbohydrates (or inorganic acids), and ammonia, nitrogen, or nitrates.

(3) Bacteria which, like the higher animals, require both organic carbon and organic nitrogen compounds. These bacteria cannot accomplish either carbohydrate or protein synthesis out of inorganic substances. Upon such principles Jensen constructed an outline of a system of classification which has many merits, although it has not been generally adopted.

One of the earlier morphologic classifications of bacteria was drawn up by Migula.[1] Migula's classification served a useful purpose for some years, and had an important influence on bacterial nomenclature and grouping. It proved, however, open to objection in many details, and the morphologic classification of bacteria has long been in an admittedly confused and unsatisfactory condition.

Along such lines a committee of the Society of American Bacteriologists[2] spent several years in studying the characterization and classification of bacterial types, and has drawn up a working classification which possesses many advantages.[3] On this system the Schizomycetes are divided into five orders: (1) Myxobacteriales, (2) Thiobacteriales, (3) Chlamydobacteriales, (4) Actinomycetales, (5) Eubacteriales. The first three are the so-called "higher bacteria" and include (1) the myxobacteria, organisms showing a pseudoplasmodial stage which passes over into a highly developed cyst-producing resting stage;[4] (2) the sulfur bacteria, organisms typically found in water containing hydrogen sulfid or other sulfur compounds and whose cells contain either granules of pure sulfur or the pigment bacterio-purpurin;[5] (3) the iron bacteria, typically water forms, usually with a sheath containing a deposit of iron oxid.[6]

[1] Migula: "System der Bacterien," Jena, 1897.

[2] C.-E. A. Winslow, Jean Broadhurst, R. E. Buchanan, Charles Krumwiede, Jr., L. A. Rogers, H. G. Smith.

[3] Journal of Bacteriology, 1920, 5, p. 191.

[4] Cf. R. Thaxter: Bot. Gaz., 1892, 17, p. 394.

[5] Cf. S. Winogradsky: "Zur Morphologie und Physiologie der Schwefelbakterien," Leipzig, 1888.

[6] Cf. H. Molisch: "Die Eisenbacterien," Jena, 1910.

The two remaining orders, (4) Actinomycetales and (5) Eubacteriales, are those with which the bacteriologist is primarily concerned. The committee's description and the artificial key proposed by them follow:

"Order Actinomycetales, Buchanan, 1917a, p. 162.[1]

"Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings, clubbed or irregular shapes. No pseudoplasmodium. No deposits of free sulphur or iron. No bacteriopurpurin. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Some species are parasitic in animals or plants. Not water forms. Complex proteins frequently required. As a rule strongly aërobic (except for some species of Actinomyces and the genera Fusiformis and Leptotrichia) and oxidative. Growth on culture-media often slow; some genera show mold-like colonies . . .

"Order Eubacteriales, Buchanan, 1917b, p. 162.[1]

"The order Eubacteriales includes the forms usually termed the true bacteria, that is, those forms which are considered least differentiated and least specialized. The cell metabolism is not primarily bound up with hydrogen sulphide or other sulphur compounds, the cells in consequence containing neither sulphur granules nor bacterio-purpurin. The cells apparently do not possess a well-organized or well-differentiated nucleus. These organisms are usually minute and spherical, rod-shaped or spiral, in most genera not producing true filaments; and rarely branching. The cells may occur singly, in chains or other groupings. They may be motile by means of flagella, or non-motile, but are never *notably* flexuous. Cell multiplication occurs always by transverse, never by longitudinal, fission. Some genera produce endospores, particularly the rod-shaped types. Conidia not observed. Chlorophyll is absent, though the cells may be pigmented. The cells may be united into gelatinous masses, but never form motile pseudoplasmodia or develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the Myxobacteriales . . .

[1] Jour. Bacteriol., 1917, 2, pp. 155–164.

"ARTIFICIAL KEY TO THE FAMILIES AND GENERA OF THE ACTINOMYCETALES AND EUBACTERIALES

"A. Typically filamentous forms ..... Actinomycetaceæ.
B. Mycelium and conidia formed ..... Actinomyces
BB. No true mycelium.
C. Cells show branching.
D. Gram-negative ..... Actinobacillus.
DD. Gram-positive ..... Erysipelothrix.
CC. Cells never branch. Gram-positive threads later fragmenting into rods ..... Leptotrichia.
"AA. Typically unicellular forms (although chains of cells may occur).
B. Spherical cells. Coccaceæ.
C. Parasitic forms. Cells in pairs, chains or irregular groups, never in packets. Generally active fermenters.
D. Cells in flattened coffee-bean-like pairs. Gram-negative ..... Neisseria.
DD. Cells not as above. Gram-positive.
E. Cells in lanceolate pairs or chains. Growth on media not abundant.
F. Cells in lanceolate pairs. Inulin generally fermented ..... Diplococcus.
FF. Cells in chains. Inulin generally not fermented ..... Streptococcus.
EE. Cells in irregular groups. Growth on media fairly vigorous. White or orange pigment. Staphylococcus.
CC. Saprophytic forms. Chains occurring in zoögleal masses in sugar solutions ..... Leuconostoc.
CCC. Saprophytic forms. Cells in irregular groups or packets, not in chains. Fermentative powers low.
D. Packets formed ..... Sarcina.
DD. No packets.
E. Yellow pigment ..... Micrococcus.
EE. Red pigment ..... Rhodococcus.
BB. Rods.
C. Curved rods. Spirillaceæ.
D. Short comma-like rods. One-three short flagella. Vibrio.
DD. Long spirals, five-twenty flagella ..... Spirillum.
CC. Straight rods.
D. No endospores.
E. Rods of irregular shape or showing branched or filamentous involution forms.
F. Animal parasites. Cells of irregular shape. Staining unevenly.
G. Acid fast ..... Mycobacterium.
GG. Not acid fast.
H. Cells elongate, fusiform ..... Fusiformis.

HH. Cells not fusiform, sometimes branching.
I. Gram-positive. Slender, sometimes clubbed rods ..... Corynebacterium.
II. Gram-negative. Rods sometimes form threads. Characteristic honey-like growth on potato .. Pfeifferella.
FF. Not animal parasites. Cells staining unevenly and with branched or filamentous forms at certain stages. Never acid fast.
G. Metabolism simple, growth processes involving oxidation of alcohol or fixation of atmospheric nitrogen (latter in symbiosis with green plants).
H. Cells minute, symbiotes in roots of leguminous plants ..... Rhizobium.
HH. Oxidizing alcohol, branching forms common ..... Acetobacter.
GG. Not as above. Proteus-like colonies.
H. Not attacking carbohydrates. Gram-positive ..... Zopfius.
HH. Fermenting glucose and sucrose. Gram-negative ..... Proteus.
EE. Regularly formed rods.
F. Metabolism simple, growth processes involving oxidation of carbon, hydrogen, or their simple compounds or the fixation of atmospheric nitrogen ..... Nitrobacteriaceæ.
G. Fixing nitrogen or oxidizing its compounds.
H. Fixing nitrogen.
Cells large; in soil ..... Azotobacter.
HH. Oxidizing nitrogen compounds.
I. Oxidizing ammonia ..... Nitrosomonas.
II. Oxidizing nitrites ..... Nitrobacter.
GG. Not as above.
H. Oxidizing hydrogen ..... Hydrogenomonas.
HH. Not as above, using simpler carbon compounds.
I. Oxidizing CO ..... Carboxydomonas.
II. Oxidizing CH_4 ..... Methanomonas.
FF. Not as above.
G. Flagella usually present, polar ..... Pseudomonadaceæ. Pseudomonas.
GG. Flagella when present peritrichic ..... Bacteriaceæ.
H. Parasitic forms showing bipolar staining. Pasteurella.
HH. Not as above.
I. Strict parasites growing only in presence of hemoglobin or ascitic fluid ..... Hemophilus.

II. Not as above.
J. Water forms producing red or violet pigment.
K. Pigment red..... Erythrobacillus.
KK. Pigment violet..... Chromobacterium.
JJ. Not as above.
K. Plant pathogens..... Erwinia.
KK. Not as above.
L. Gram-positive, forming large amount of acid from carbohydrates and sometimes CO_2, but no H_2..... Lactobacillus.
LL. Gram-negative, forming H_2 as well as CO_2 if gas is produced..... Bacterium.
DD. Endospores present, Bacillaceæ.
E. Aërobes..... Bacillus.
EE. Anaërobes..... Clostridium."

Such a classification crystallizes much of the current practice in the grouping of bacteria, and while the introduction of new generic names presents some real difficulties, it is believed that in the long run the use of terms like Neisseria for the gonococcus-meningococcus group and Hemophilus for the hemophilic bacilli will make for clearness and simplicity.

Among the spherical bacteria, differences in the mode of grouping of the cells have given origin to certain names—as *streptococcus*, *staphylococcus* or *micrococcus*, and *sarcina*—that have been used as the names of genera, but no such generally useful distinctions have been found among the rod or the spiral bacteria. Consequently a term like Bacillus has come to serve as the genus name for an enormous number of bacteria. Some of the bacteria included under the name Bacillus are very different morphologically and physiologically.[1] Recognizing the limitations of even the more satisfactory morphologic classifications, it has been a common custom for bacteriologists to class together for purposes of study those bacteria having in common certain well-defined and salient characteristics. Such a large and well-defined group of bacilli as the colon-typhoid group might be conveniently treated as an independent genus, and the same can be said of other groups of bacteria.

Nomenclature.—The present nomenclature of bacteriology may be criticized on two grounds: first, as already pointed out, for the unwieldy size that certain "genera" have been allowed to assume;

[1] *E. g.*, Bacillus subtilis, B. typhosus, B. tetani, B. prodigiosus, B. diphtheriæ.

and, second, for the haphazard way in which trinomial and even quadrinomial names have been bestowed. Such names can be properly employed only with reference to subspecies or varieties; and designations like B. coli communis, Granulobacillus saccharobutyricus mobilis non-liquefaciens, and Micrococcus acidi paralactici liquefaciens Halensi, are both cumbersome and unscientific. The use of a single genus name for a multitude of organisms is, in fact, responsible for the tendency toward trinomial nomenclature, and the remedy for both conditions would seem to lie in the abandonment of such a term as Bacillus for the name of a genus and the frank establishment of new genera on the basis of physiologic characters, such, for example, as distinguish the colon-typhoid group or the diphtheria group of bacilli. Until some such reform in nomenclature is brought about the names used to designate different kinds of bacteria will fail to make clear the group relationships which undoubtedly exist, and will continue to be a stumbling-block to all students of the subject. Buchanan[1] has subjected the nomenclature of the bacteria to a thorough study and revision on the basis of the International Rules for Botanical Nomenclature. The work of the Committee of the Society of American Bacteriologists, already referred to, is a promising beginning in the direction of nomenclatory change and is utilized as far as practicable in this book.[2]

Variations.—Like other living organisms, bacteria of the same species are not all precisely similar: races, strains, or individuals are found which differ more or less widely from the parent form. The term variation as used in biology signifies not the manifestation of certain apparently novel qualities that appear uniformly when organisms are placed under new conditions of life (latent characteristics or environmental modifications), but a constant difference in one or more features when the individual in question is compared under the same circumstances with one or more organisms of similar descent. Unlike the modifications due to environmental influence, variations are essentially dependent on elements intrinsic, not extrinsic, to the organism.[3] To illustrate: the ability of actino-

[1] Buchanan: General Systematic Bacteriology, Baltimore, 1925, pp. 597.

[2] A still more elaborate and far-reaching change is introduced in Bergey's Manual of Determinative Bacteriology (2nd Edition, Baltimore, 1925, pp. 462).

[3] This, however, is not to be taken to imply that the property of variation itself may not be affected by a change in environment.

myces to produce club forms is manifested only in the presence of animal fluids, but the production of the clubs is not an instance of true variation; a culture of actinomyces that did not produce clubs under these conditions would be properly regarded as a variant.

Two kinds of variability are commonly recognized: (1) Variations of the ordinary "fluctuating" type, which are distributed more or less systematically about a modal condition and may be grouped in a frequency curve or frequency polygon. As the term indicates, fluctuating variability swings to and fro, oscillating around an average type. The familiar deviations in human height exemplify this kind of variation. It is supposed by de Vries[1] that fluctuations remain fixed within certain limits, and that the accumulation of fluctuating variations can never give rise to a new quality in an organism or bring about the formation of a new species. (2) "Discontinuous variations," "sports," or "mutations," on the other hand, are supposed to be perfectly definite changes which arise suddenly without the interposition of a series of intermediate forms, and having once appeared, are permanent, and show no tendency to return to the mean of the parent form.

In general, bacteriologists will not hesitate to classify the variations with which they are most familiar as those of the fluctuating type. The number of them is practically infinite, and, especially in some groups, there is little doubt that they have been given too great classificatory value, as compared with precisely similar deviations in higher forms of life. At the same time these biologically trivial differences are often of practical importance, as in the study of pathogenicity or virulence.

A very characteristic and common form of variation among bacteria consists in the loss of some quality possessed by the organism when first taken under observation. The disappearance of the power to liquefy gelatin, as in the Proteus group, or to produce pigment, as in the case of B. violaceus, and the loss of virulence, as in many pathogenic forms, are every-day occurrences in the bacteriologic laboratory. The term "retrograde variety" would seem appropriate for these forms if it had not already been applied by de Vries to mutations of a retrogressive character. In his terminology, both elementary species (the progressive forms) and retrograde varieties are supposed to have originated as sudden mutations.

[1] De Vries: "Mutationtheorie," 2 v., Leipzig, 1901–1903.

Now, so far as many of the more commonly observed retrogressions in bacteria are concerned, there can be no question that they arise from the accumulation of fluctuating variations, and not in any sudden and unexpected fashion. Minute divergences from the first culture grow more and more pronounced in each succeeding cultural transfer, and finally the once conspicuous and taxonomically important character may disappear altogether. Such retrogression, or, as it is often styled, degeneration, is no rare occurrence; it takes place with great uniformity, and follows definite and similar lines in a number of widely different organisms. In cultures, many totally unrelated organisms lose, by degrees, virulence, gelatinolytic power, or capacity for chromogenesis. Such a change goes on steadily and occurs in nearly all strains coming under observation. The varieties so formed grow luxuriantly and are perfectly stable under the conditions in which they are ordinarily kept; in fact, often they cannot be made to regain their lost property, even by successive transfers upon media considered to be especially favorable for the manifestation of the quality in question; they are spoken of as hopelessly degenerate. This is the case, for instance, with many strains of B. fluorescens as regards pigment production. Qualities once lost, however, may under certain conditions be regained, as shown not only in the reacquisition of chromogenic power, but also in the reassumption of virulence by attenuated pathogenic races.

There is now no doubt that true mutations occur among bacteria as among the higher forms of life. Barber,[1] for example, has described an instance in a strain of the dysentery bacillus in which the variation occurred suddenly and fully formed; it appeared in a relatively small number of individuals, was not adaptive, and the new characteristics were transmissible to offspring through many generations. A similar instance has been observed in B. coli,[2] in which cells with the power of fermenting saccharose and raffinose suddenly appeared in a culture (from a single cell) that had not previously possessed this power. The parent strains and the mutating strains have each maintained their separate characteristics for over four years and during some hundreds of test-tube transfers.

Several writers have also reported the occurrence of secondary colonies or papillæ (Ger. *Knöpfe*) on colonies of varieties of B. coli

[1] M. A. Barber: Philippine Jour. of Science, 1913, 8, p. 539.

[2] Jordan: Proc. Nat. Acad. of Sci., 1915, 1, p. 160.

grown on certain carbohydrate media. These papillæ are composed of cells having the power of fermenting the carbohydrate with which they are in contact, a power which the main colony lacks. The appearance of these secondary colonies is interpreted by some writers as mutation, by others as adaptation.[1]

Connected with the variability of bacteria is their remarkable plasticity or adaptability to diverse conditions of life. By a series of inoculations or transfers it is possible so to alter bacteria that qualities originally present are sometimes accentuated, sometimes abolished. Bacteria may become adapted to very high temperature, to growth in the presence of antiseptics, and even to multiplication in strongly bactericidal sera. Such modifications, as a rule, are gradually acquired and gradually lost. Pathogenic bacteria, like other parasites, may become so strictly adapted to life in the tissues of a given animal species that they neither grow readily in artificial culture-media nor in the bodies of animals closely related to the particular host. This seems, for example, to be the case with the leprosy bacillus, which, so far as known, is not able to grow anywhere except in the body of man and possibly the anthropoid apes. Theobald Smith[2] has made the important suggestion that bacteria of great pathogenic power should be looked upon as incompletely adapted parasites that have not yet succeeded in establishing an equilibrium between themselves and their host. The less complete the adaptation, the more virulent the disease produced. This would explain the tendency of long-established diseases to decrease in severity at the same time that they are becoming more frequent.

According to Rosenow[3] an interesting example of bacterial specialization occurs in the so-called elective localization of streptococci and other organisms. Certain strains of bacteria have been thought to show a preference or "affinity" for certain tissues, so that, for example, certain epidemics of throat infection, due to streptococci, are frequently complicated by sinusitis, while others due to very similar streptococci lack this complication. Rosenow

[1] M. Neisser: Centralbl. f. Bakt., I, Ref., 1906, 38, Beiheft, p. 98; Massini: Arch. f. Hyg., 1907, 61, p. 250; Bencke: Ztsch. f. ind. Abstammungs- u. Vererbungslehre, 1909, 2, p. 215; Burri: Centralbl. f. Bakt., II, 1910, 28, p. 321; Baerthlein: Arb. a. d. k. Ges., 1912, 40, p. 433.

[2] Smith, Theobald: Proceedings of Congress of Arts and Science, St. Louis, 1904, 5, pp. 219–239.

[3] Rosenow: Jour. Amer. Med. Assoc., 1915, 65, p. 1687.

found that 14 strains of streptococci from appendicitis produced lesions in the appendix in 68 per cent. of the rabbits injected, whereas an average of only 5 per cent. of lesions in the appendix were produced by streptococci from other sources. Irons[1] has observed a similar elective affinity of streptococci in iritis. Although seemingly supported by a large amount of experimental work the theory of elective localization has not been generally accepted.

The question whether adaptation to a particular tissue or culture-medium or to a high or low temperature, is due to the selection of fluctuating variations or of mutations is one to which the existing evidence does not give a conclusive answer. On the whole, the available data indicate that change takes place by gradual progression rather than by sudden leaps. How far modification of individual cells and transmission of such modifications to the daughter cells is responsible for the observed conditions has not been determined.[2]

A singular development in the study of bacterial varieties has been the discovery of the existence of so-called "immunological varieties," that is to say, strains apparently possessing identical cultural characters, but differing in agglutinative or other immunity reactions. Streptococci, gonococci, tetanus bacilli, and other organisms, especially pneumococci, have been divided into various serological types, based on the reactions shown by various strains to the sera of immunized animals. Thus the serum of an animal immunized to Type I pneumococcus will agglutinate strains obtained from certain cases of pneumonia in widely separated localities, but will not agglutinate other strains derived from other cases and belonging to Types II, III and IV. The origin and the significance of these immunological varieties have not yet been determined.

[1] Irons: Jour. Infect. Dis., 1916, 18, p. 315.

[2] A good summary of the early work on variation in bacteria has been given by Pringsheim, "Die Variabilität niederer Organismen," Berlin, 1910; for a more modern discussion see Hadley: Jour. Infect. Dis., 1927, 40, p. 1.

CHAPTER VII

BACTERIA AND DISEASE IN ANIMAL ORGANISMS

Theories of Disease.—In order to understand in some degree the influence of bacteriology upon medicine, it is worth while to recall the more important theories and conceptions regarding disease that have been held by the human race.

Probably one of the earliest notions of the cause of disease was a belief that an evil spirit or demon entered into or possessed the body of a man and there wrought various ills. This at least is the belief still widely prevailing at the present day among savage tribes, which represent in many particulars an early stage of culture, and one through which the ancestors of modern civilized man probably passed. "The possessed man, tossed and shaken in fever, pained and wrenched as though some live creature were tearing or twisting him within, . . . rationally finds a personal spiritual cause for his sufferings. In hideous dreams he may even see the very ghost or nightmare fiend that plagues him . . . This is the savage theory of demoniacal possession, which has been for ages, and still remains, the dominant theory of disease and inspiration among the lower races. It is obviously based on an animistic interpretation, most genuine and rational in its proper place in man's intellectual history, of the actual symptoms of the cases."[1] This animistic or demonistic conception of disease still finds expression in the practices of the medicine-men or wizards of many savage peoples. Granting that a spirit is the cause of a disease, the logical proceeding is to induce the spirit to leave the body of the patient. Two modes of treatment are possible: the spirit may be lured out by propitiatory sacrifices, fair promises, or other conciliatory measures, or he may be forcibly evicted by powerful charms, by the beating of tom-toms, or by pummelling the body of the patient. Examples of the methods of treatment used by these two schools of medical practice may be readily found among travellers' descriptions of customs which prevail today among many primitive and

[1] Tylor: "Anthropology," New York, 1904.

half-civilized peoples. As regards one large class of diseased persons, the insane, the theory of demoniacal possession has remained current up to comparatively recent times.

When the advance of natural knowledge brought a larger measure of understanding of the structure and functions of the human body, a new and semi-scientific theory of disease sprang into being and attained worldwide influence. The Hippocratic theory of disease, as it was called after its founder, Hippocrates, "the Father of Medicine," was, in fact, the dominant theory all through the middle ages, and still colors much medical thought and practice. According to this celebrated theory, the body contains four humors: blood, phlegm, yellow bile, and black bile. Health consists in a proper mixture of these four humors; disease, in an improper mixture. The doctrine of temperaments arose as an outgrowth of the Hippocratic theory: according as one or another humor preponderated a man was said to be of a sanguine, phlegmatic, or melancholic temperament. In acute disease the humors went through a regular process, being first of all *crude*, then passing through *coction*, or digestion, and finally being expelled by resolution or *crisis* through one of the natural channels of the body. The efforts of physicians were to be directed toward keeping the humors in their proper relations one to another, for if the normal delicate balance became disturbed, the most serious results might follow. This ingenious conception of disease held almost undisputed sway for a long period, and was hardly seriously questioned until the seventeenth century. In the seventeenth and eighteenth centuries a number of novel theories were propounded, but were of little aid to medical progress: in fact, many of the substitutes proposed for the Hippocratic theory were more complex and more mystical than the belief they were intended to supplant. Disease was "an intestine movement of particles," it was an attempt of nature to eliminate morbific matter, a want of "tone," a deficiency of stimulus, and so on. The theory of this class that lasted longest and had the greatest effect upon medical practice was the so-called theory of homeopathy. The definition of disease given by Hahnemann, the founder of this school, is an index of the greater part of his teachings. "Disease," said this writer, "is a spiritual dynamic derangement of a spiritual vital principle." His theory of potentiality or dynamization maintained that medicines gained in strength by diluting, if the

dilution was accompanied by shaking; the potency of some drugs was also supposed to be increased by pounding. Acting on this doctrine, Hahnemann ordered his original tinctures to be reduced in strength to one-fiftieth; these first dilutions again to one-fiftieth; and so on even to the thirtieth dilution, which he himself used by preference and to which he ascribed the highest "potentiality." An interesting outcome of this procedure was the fact that while such highly "potentialized" drugs could not be rationally supposed to have any physiologic effects, their administration to a patient was sometimes followed by more favorable results than was the more "regular" administration of drugs. A mode of treatment which consisted essentially in giving no medicine was sometimes as successful or more successful than the ordinary procedure. In a word, homeopathy, although theoretically ridiculous, did much to reform the custom of indiscriminate giving of drugs in large doses.

Amid the vagueness and confusion of such half mystical hypotheses as homeopathy, emerged the more tangible and definite germ theory of disease. As already pointed out, the germ theory of disease was the legitimate offspring of the germ theory of fermentation, and owed its origin to the memorable investigations of Louis Pasteur. The belief that the so-called infectious diseases are caused not by any enraged and revengeful spirit, not by any improper mixture of four humors, not by any spiritual dynamic derangement, but by small living plants and animals is now securely established. As regards such diseases as diphtheria, tuberculosis, and Asiatic cholera, this belief is no longer a hypothesis, but is based on indisputable fact. In respect to some other diseases, like smallpox, hydrophobia, and influenza, while no specific micro-organism has been established as the cause, there can be little doubt that the germ theory of causation furnishes the most reasonable, consistent, and probable explanation of the nature of these infections.

Pathogenesis

The conception of a pathogenic micro-organism is a relative, not an absolute, one; that is to say, no microbe is known that is capable under all conditions of producing disease in all animals. As a rule, a pathogenic bacterium is limited in its activities to a small number of hosts; bacteria pathogenic for animals are not ordinarily pathogenic for plants; very few of the bacteria that can infect mam-

mals are also pathogenic for cold-blooded animals; some are even restricted to the tissues of a single species.

The power of a microbe to produce morbid effects or changes depends, therefore, as much upon the nature of the host as upon its own characteristics. A bacterium that is pathogenic for one animal species may be harmless for another: the typhoid bacillus, when swallowed by man, can produce a serious, often mortal, illness; when fed to cattle, it produces no effect. As a consequence, no sharp line can be drawn between pathogenic and non-pathogenic micro-organisms. One of the common, typically saprophytic bacteria, the ubiquitous hay bacillus (B. subtilis), which is found almost universally in air, water, and soil, is capable under certain conditions of giving rise to a serious affection of the human eye.

Factors Affecting the Host.—The ability of a micro-organism to produce disease in individuals of a particular race or species may be modified by a number of general factors that predispose individuals to infection or endow them with resistance. The conditions that determine whether or not a microbe can bring about infection are numerous and varied. A few illustrations will suffice. The *age* of an individual is often of great importance. Experiments have shown that, while the adults of certain animal species are resistant to inoculation with a particular germ, the young of the same species may succumb. The existence in the human race of a number of "children's diseases," which are not only more common, but more fatal, among children than among adults, is evidence to the same effect.[1] *Hunger* and *thirst* predispose to infection. If pigeons are kept on a low diet before, or just after, inoculation with anthrax bacilli, they die, although under normal conditions these birds are naturally immune to anthrax. Animals deprived of water also lose their natural resistance to anthrax inoculation. An unsuitable diet, as the substitution of bread and milk for meat, has the same effect. Excessive *fatigue* will predispose to infection. The normal white rat is highly insusceptible to anthrax, but when exhausted by work in a treadmill, becomes very susceptible. *Exposure to extremes of heat and cold* is well known to depress resistance to infection. This is shown by one of Pasteur's classic experiments, in which he rend-

[1] In a series of nearly 70,000 cases of scarlet fever admitted to the Hospitals of the Metropolitan Asylum Board in London the case mortality per cent. was 18.2 among those under five years of age and 2.8 among those from twenty to twenty-five years.

ered the naturally resistant hen susceptible to anthrax by chilling it with cold water. The prevalence of pneumonia in man in those months of the year when the influence of cold upon the human organism is most felt affords another illustration of the same fact. Frogs, which are immune to anthrax at ordinary room temperature, quickly die after anthrax inoculation if placed at a temperature of 25° to 35° C. Wasting diseases, like diabetes and typhoid fever, favor a secondary invasion of the tissues by micro-organisms, especially those belonging to the group of pyogenic bacteria. In diseases such as measles and smallpox the weakening of resistance due to the primary specific infectious agent is followed by the invasion of the tissues by streptococci, which are responsible for a large part of the injury done to the organism in these maladies.

In addition to these and other predisposing factors which affect the general resistance of the whole organism, there are causes which influence the resistance of particular organs or groups of tissues. Local susceptibility may be increased by defective blood-supply, by rapid growth, by mechanical injury or trauma, and other factors. When streptococci are injected into the circulation of a perfectly healthy rabbit, they rarely settle on the valves of the heart, but if the aortic cusps of the heart have been injured prior to infection, the cocci gain a foothold there and set up an ulcerative endocarditis. The special liability of the bones and joints of young children to tuberculosis and suppurative affections is a well-known instance of local susceptibility. The organ or tissue that offers temporarily or constantly the point of least resistance is in each case the one to be attacked. The great danger of infection of the human mother at childbirth, unless every care be taken to prevent the access of bacteria to the uterine cavity, typifies the peculiar peril that arises when there is a concurrence of a severe local injury and a generally weakened condition.

Factors Affecting the Microbe.—Not only do the nature and state of the host play an important part in determining the occurrence of infection, but the conditions influencing the infecting agent itself are also of great importance. The *virulence* of a micro-organism, that is, its power of growing in the body and producing injury, varies just as does the susceptibility of the host. Certain races or strains of bacteria occur that are characterized by a high or low degree of virulence, and there is no doubt that the varying severity

of cases of infectious disease is due in part to differences in the virulence of the attacking germ as well as to differences in the resistance of individual hosts. Experimentally, for example, there is a great difference in pathogenicity for guinea-pigs between various strains of pneumococci isolated from cases of lobar pneumonia. The virulence of a microbe for a particular species may usually be increased by successive animal inoculations, the microbe recovered from one animal being inoculated into another, and so on. This occurs, for example, when pneumococci that are avirulent for guinea pigs are successively passed through a series of these animals. The increased virulence acquired as a result of this method of animal passage does not necessarily obtain in the case of other host species. The virulence of certain streptococci for mice is increased by passage through the bodies of these animals, but at the same time the virulence for rabbits is diminished. Virulence may be decreased in a variety of ways, as, for example, by growth at high temperature or in the presence either of antiseptics or of the serum from an animal which is immune to the microbe. A strain that has been weakened in virulence is said to be *attenuated*. An attenuated culture may be vigorous in other respects. Tubercle bacilli when first isolated from the mammalian body are usually quite virulent, but grow feebly on artificial culture media; after some months' cultivation they grow more luxuriantly, but have lost in virulence.

The production of infection depends likewise upon the *number* of bacteria introduced into the body. An organism will often cope successfully with a small number of bacteria when the ingress of a larger number will cause a fatal disease. Ordinarily the injection into an animal like a rabbit of a few dozen or a few hundred bacteria has little effect, although it is said that inoculation with a single virulent anthrax bacillus will prove fatal to the very susceptible white mouse. In every case the number of bacteria necessary to produce infection will depend both upon the virulence of the culture and upon the racial and individual susceptibility of the host. Indeed, a division of pathogenesis into microbic virulence and host susceptibility is really artificial. The characteristics of the microbe and of the host together determine the outcome of an infection.

Routes of Infection.—The particular tissues with which a germ first comes in contact, that is, its path of entrance into the body, often exercises a decisive influence upon the production of infection.

Injection directly into the circulation (intravenously) will many times bring about infection, when subcutaneous injection of the same number and kind of germs fails to produce any effect. Some pathogenic bacteria may be taken into the alimentary tract with impunity, while a fatal infection will ensue if inoculation be made into the peritoneum; typhoid bacilli, for example, may be fed to an adult rabbit in large numbers without causing death or even serious illness, whereas intraperitoneal inoculation provokes a fatal infection. On the other hand, there is evidence that the spirillum of Asiatic cholera is much more pathogenic for man when swallowed than when introduced under the skin. Organs or tissues of weak vitality constitute a break in the line of resistance through which various bacteria may find their way: thus the human tonsils are a much-used portal of entry for pathogenic microbes. Vulnerability to a particular channel of infection may be incurred by mechanical injury or deranged metabolism: gastric disorders which alter the normal acidity of the stomach juices seem to predispose to infection with Asiatic cholera; inhalation of dust-particles in themselves not infectious is well known to increase the liability to pulmonary tuberculosis.

Bacteremia and Toxemia.—A distinction, in some respects important, is made between those infections in which bacteria become widely disseminated throughout the body and those in which they remain quite strictly localized. In the latter case general or constitutional symptoms may be produced through the action of soluble poisons which are produced by the bacteria, absorbed at the point of their production and carried to distant organs. Diphtheria and tetanus are primarily toxemic diseases. In tetanus the bacilli are not found in the blood or internal organs; the local reaction caused by their presence is itself slight and often insignificant, and lesions have not been detected at the site of inoculation; but the toxin makes its way slowly along the nerves to the central nervous system, where it produces profound disturbance.

At the opposite pole from this condition are those general invasions of the host in which bacteria multiply abundantly in the blood or tissues and are sometimes found on autopsy in large numbers in the capillaries in various organs. This condition of bacteremia is fairly common; in man bacteria are present in the blood not only in the so-called "blood-poisoning" maladies, but in diseases

like pneumonia and typhoid fever. In some infections of lower animals, such as anthrax, the multiplication of bacteria in the body is the most prominent feature of the disease. It is possible, however, that micro-organisms multiplying in the body may produce poisons even though no poisons can be detected in artificial cultures outside of the body, and hence that in effect no strict dividing-line separates bacteremia from toxemia. Both multiplication and poison-production accompany infection as a rule, sometimes one factor being more prominent, sometimes the other.

The distribution of micro-organisms throughout the body varies greatly. In the toxemic diseases like diphtheria and tetanus the bacteria remain, as a rule, strictly localized. In others they are at first localized, but spread by continuous extension, as in erysipelas and many other infections. In still others they are borne in the lymph or blood-stream to a greater or less distance from the primary focus, and set up a secondary focus, or secondary foci, sometimes in remote organs. This is the so-called spread by metastasis. Certain micro-organisms for unknown reasons settle in particular organs much more frequently than in others.

The term *pyemia*, or metastatic infection, is commonly applied to a condition in which secondary foci of suppuration appear, and multiple abscesses are formed in the internal organs and generally throughout the body. *Septicemia*, in the bacteriologic use of the term, refers to the presence or multiplication of micro-organisms within the blood; in this state the bacteria are found abundantly in the capillaries. It is here used in the same sense as *bacteremia*. The terms bacteremia and septicemia are applied by some writers solely to the presence of bacteria in the blood; as used by others they are limited to conditions showing multiplication of bacteria in the blood. There is no general agreement in respect to the latter restriction. The term septicemia is also used in surgery more narrowly as applying to a condition in which the bacteria of suppuration invade the blood, but no abscesses are produced in the organs. Broadly speaking, pyemia is a particular variety or manifestation of bacteremia.

Mixed and Secondary Infections.—Physicians have long been aware that an individual might be attacked at one time by two or more infections. Diphtheria and scarlet fever, syphilis and gonorrhea, pneumonia (due to the pneumococcus) and typhoid fever, are

combinations by no means unknown. It is possible that in some cases the different infections may originate nearly simultaneously, but such an occurrence is probably not common. Usually one infection precedes another, and the second is very frequently a more or less direct outcome of the first. Infection with certain micro-organisms predisposes to secondary infection with the pneumococcus; acute tuberculosis may develop during an attack of measles; streptococcus invasion of the lung tissues is common in pulmonary tuberculosis. The secondary invader is commonly present in the host, apparently incapable of initiating an infection until the host defenses are weakened by the primary disease. Certain micro-organisms that can cause primary infection are also frequently found as secondary invaders. Pneumococci and streptococci are preëminent in this respect, and show a remarkable capacity for invading the body in the wake of other micro-organisms.

Mixed infections of a somewhat different sort are those in which the principal pathogenic organism is accompanied by auxiliary microbes, or, as some French bacteriologists have called them, accomplices, which by their presence influence the virulence of the chief infectious agent without themselves taking any very active part in the infectious process. The aërobic bacilli which usually enter a wound along with tetanus bacilli probably facilitate the growth and activity of the latter and thus aid in producing infection and toxemia. In other cases two or more pathogenic organisms may aid one another in weakening or breaking down the natural defenses. Diphtheria bacilli in the throat are often accompanied by streptococci, and there is reason to believe that such a mixed infection is more severe than an infection with diphtheria bacilli alone.

The External Defenses of the Organism.—*The Skin.*—As a rule, the unbroken skin presents a more or less impassable barrier to micro-organisms. Virulent bacteria, especially staphylococci and streptococci, are found normally on the skin or between the superficial horny cells, but are not able ordinarily to penetrate deep into the tissues unless favored by some cutaneous injury, such as a wound or burn. The ducts of the sweat-glands and the hair-follicles are, however, vulnerable points, and experiments have shown that it is possible for germs to make their way to the underlying tissues through these channels. Such an entrance through the uninjured skin must be regarded as exceptional. Even if the outer defenses

are passed, the subcutaneous connective tissues present obstacles to further invasion, partly perhaps by mechanical means through rapid formation of new connective tissue, partly through the bactericidal properties of the lymph and the action of phagocytes.

The Mucous Membranes.—While the moist condition of mucous surfaces is favorable to bacterial multiplication, the constant removal of the layer of mucus tends to prevent bacteria from gaining permanent lodgment. The mucus itself has only a slight bactericidal power. The conjunctiva is protected both by irrigation with the mildly germicidal lacrimal secretion and by the action of the eyelashes and eyelids, although these mechanical defenses are frequently overcome by various pathogenic bacteria. The mucous membranes of the nasal cavities are protected to some extent against bacteria by the tortuous nature of the nasal passages, by the mechanical barriers interposed by the hairs, and also by the action of the ciliated epithelium, which sweeps mucus and dust particles outward. Nevertheless, streptococci and other organisms frequently use this path of entrance. The healthy human mouth presents a highly favorable environment for the development of bacteria, and it is not surprising that upwards of fifty kinds of bacteria have been described as occurring in this locality. Pneumococci and streptococci are probably constantly present, and meningococci and diphtheria bacilli are also often found in the mouth of persons apparently in perfect health, but who have been more or less intimately associated with patients or convalescents. The saliva is feebly germicidal, and when secreted in normal quantities serves as a wash.

The Lungs.—The dust particles in a current of air cling to the moist surfaces with which they come in contact; in this way air becomes largely freed from bacteria in the upper respiratory passages. Those bacteria that pass the larynx are caught in the bronchi, and few reach the ultimate ramifications of the bronchioles. Both the fixed alveolar epithelial cells and the wandering leukocytes that enter the bronchioles and sacs have been observed to take up bacteria. Under some conditions, not clearly understood, the natural defenses of the lungs are broken down and infection of these organs seems to occur rather readily.

The Stomach.—The normal gastric juice is decidedly unfavorable to the growth of bacteria, a property due to the hydrochloric acid it

contains; this is doubtless the reason that the stomach is so free from infection. The action of the gastric juice does not, however, prevent the frequent passage of swallowed pathogenic germs into the intestines, perhaps because such germs are frequently embedded in solid particles of food which protect them from the bactericidal action of the stomach fluid, perhaps because the gastric juice restrains development but does not kill. Tetanus and diphtheria toxins are rendered harmless by gastric juice, but a rare toxin sometimes found in food stuffs (produced by Cl. botulinum, p. 422) is not affected.

The Intestines.—The intestinal secretions have, on the whole, little restraining power over bacterial multiplication, although the bile has feeble germicidal properties. The peristaltic movements of the stomach and intestines may afford, it is thought, some degree of protection against invasion. The number of bacteria in the intestinal contents increases from the duodenum onward to the colon. Bacteria of more or less pathogenic power, such as streptococci and B. coli, occur in the healthy intestinal tract, often in large numbers. As long as the tissues are perfectly normal these organisms are quite harmless, but, like the pathogenic bacteria of the mouth and upper air-passages, their invasive power is increased by a diminution in the resistance of the tissues with which they are in contact. The intestinal disturbances in children under the influence of continued hot weather may be in large part due to the normal bacterial inhabitants of the intestines, rather than to any specific infection. On the other hand, well-known pathogenic bacteria like the typhoid bacillus and the cholera spirillum may exist in the human intestine, possibly multiply there to a limited degree, and be ejected in the feces without having induced disease.

The Transmission of Infection.—Some infectious diseases are caused by microbes which are naturally saprophytic and which enter the animal body accidentally, as it were, rather than from choice of it as a culture-medium. Such, for example, seems to be the case with tetanus, the bacillus of which is not a parasite by conviction, but lives habitually as a saprophyte in soil and in the intestinal contents of the horse and some other animals. The other pathogenic anaërobes of the soil are also essentially saprophytic. As a rule, however, those bacteria that produce disease are more or less closely adapted to a parasitic existence, and pass from one

animal body to another with only a relatively brief sojourn in the external world. The large majority of the bacteria causing infection in man are able, under ordinary conditions, to survive only for a very limited period apart from the human body. Hence the transmission of infection is in most cases dependent upon contact, either directly with an infected individual, or with material recently cast off from the body of such a person. Important differences exist in the resistance of pathogenic micro-organisms to the influences of external nature. The gonococcus, for example, dies off very quickly; the pneumococcus and the cholera spirillum are somewhat more resistant; while the tubercle bacillus and the typhoid bacillus are fairly hardy. Multiplication of pathogenic germs outside of the animal body, barring the soil anaërobes, occurs only in a few cases, and under rather exceptional circumstances, as when diphtheria or typhoid bacilli find their way into milk.

From the point of view of preventive medicine it is especially important to note that pathogenic bacteria may exist for a long time in or upon the body of well individuals. After recovery from typhoid fever, typhoid bacilli may continue to be discharged from the bowel or bladder for months or even years. Similarly, convalescents from diphtheria may harbor virulent diphtheria bacilli in their throats for long periods. It is also true that persons who have been in contact with infected individuals, although themselves remaining healthy, may be the carriers of disease germs. Certain bacteria may be thus transmitted from a patient to another individual through the medium of a person who is himself unaffected. There is reason to believe that the living carrier of disease germs as a principal or an intermediary is a highly potent factor in disseminating disease.

The path by which a disease germ leaves the body is influential in determining the route of infection. The typhoid germ frequently passes into sewers, makes its more or less devious way into a river, lake or spring, and is perhaps eventually swallowed at a distant point. Diphtheria bacilli may be left by a child's lips on the edge of the school drinking-cup and so cause the infection of a playmate. Tubercle bacilli may be inhaled in the infectious droplets discharged by a consumptive in the act of sneezing or coughing. The infections of known origin, for the most part, are caused by germs thrown off from the mouth, from the intestines, from cuta-

neous sores, and in the genito-urinary secretions. In certain infections, not yet elucidated, such as the "acute exanthemata" (smallpox, chickenpox, etc.), the infectious agent is possibly contained in scaled-off epithelial cells as well as in discharges from the throat and nose.

CHAPTER VIII

IMMUNITY AND BODY RESISTANCE TO DISEASE

Antigens and Antibodies
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  Individual
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  Active
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The Mechanism of Immunity
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  (d) Anaphylaxis or Hypersensitiveness—Allergy—Protein Sensitization

Immunity, like pathogenicity, is a relative tĕrm; all living organisms, at least all the higher forms, are susceptible under some conditions to some kind of parasitic invasion.

On the other hand, some degree of resistance against parasitic attack seems to be manifested by all animals and plants. In certain cases the defense is so effective that bacteria and other parasitic micro-organisms rarely invade the body under natural con-

ditions. The wild carnivora, for example, are probably practically exempt from bacterial infections. The cat and the dog, as is well known in bacteriologic laboratories, show, as a rule, a high degree of resistance to inoculation with bacteria that are highly pathogenic for other animals. In some animals, such as the guinea-pig, infection occurs more readily. Man is susceptible to infection with a great variety of micro-organisms, certain of which possess little or no pathogenic power for any other animals, and is immune to many microbes that are highly pathogenic for certain lower forms.

Resistance to bacterial infection is often an inborn quality of a race or an individual. Such resistance is termed *natural immunity*, and is the converse of *natural susceptibility*. A state of natural susceptibility may be transformed by various causes into a condition of greater or less resistance, commonly designated as *acquired immunity*. Most civilized men are born naturally susceptible to smallpox, but acquire immunity during their individual lifetime either by vaccination or by an attack of the disease.

There is reason to believe that the nature of the defense set up by the organism is not the same in all cases, and that natural immunity, in particular, is often due to an entirely different set of factors from acquired immunity.

Antigens and Antibodies

When bacteria and other parasites invade the tissues they evoke certain remarkable reactions in the body of their host. These reactions are now known to belong to a group of fundamentally similar biological phenomena, the phenomena of antibody formation. The introduction into the animal body of foreign protoplasm, whether bacterial cells, red blood-cells, spermatozoa, or snake venom, gives rise to substances in the blood or tissue of the host, which have a specific action and may respectively neutralize the venom, cause disintegration of the spermatozoa, hemolyze the red blood-cells, and kill the specific bacterium. The blood or tissue having this antagonistic power is said to contain *antibodies* and, conversely, those substances capable of giving rise to antibodies in the animal body are called *antigens*. Only one class of chemical substances—the proteins—have been certainly proved to be antigenic. The evidence presented to show that certain lipoids and glucosides possess antigenic properties is not conclusive.

Natural Immunity

Natural immunity sometimes depends upon the simple fact that a micro-organism which finds favorable conditions for multiplication in one species of animal meets with unsuitable conditions in another species. Profound metabolic differences, such as those between warm-blooded and cold-blooded animals, are in themselves sufficient to account for much so-called natural immunity. In general, invertebrates are not attacked by parasites that invade the vertebrate body, and the lower vertebrates (frogs, fish, reptiles) are not affected by inoculation with the various bacteria pathogenic for birds and mammals. This is quite in keeping with the well-known physiologic and toxicologic differences between the various animal groups. Strychnin, which is so powerful a poison for vertebrate animals, has little effect upon protozoa, and quinin may be fatal to the malarial parasite at the same time that it exerts but a slight and temporary effect upon the human organism. The influence of body temperature upon infection is shown in the case of tetanus: many cold-blooded animals not normally susceptible to tetanus succumb to infection with the tetanus bacillus or to intoxication with its toxin when they are kept in a warm chamber. Flesh-eating animals, as a rule, are less prone to infection than herbivora, perhaps because of the difference in metabolism accompanying the difference in food.

Closely related *races* and *species* of animals sometimes display, one a natural immunity, another a natural susceptibility, to the same infecting agent. Field-mice are highly susceptible to glanders, house-mice almost completely immune. Jersey cows are more liable to tuberculosis than Holsteins, and Yorkshire swine are more resistant to swine erysipelas than some other porcine breeds. In man the immunity against particular diseases once thought to be possessed by certain races is not so marked as formerly supposed. No race of mankind seems to possess absolute immunity toward any human disease; in fact, such differences as are observed seem to be due very largely to differences in the opportunities for infection such as arise from diverse habits and pursuits. Yet there are some evidences that certain races are more, and others less, susceptible to tuberculosis, influenza and other infections.

Individual differences in the natural power of resistance in man come to light in the experience of every physician. Members

of the same family exposed at the same time to the same possibility of infection show greatly varying susceptibilities. Variations in degree of resistance to the suppurative infection of slight wounds and scratches are especially common and well known. It is impossible to eliminate in every case of natural infection the source of error due to differences in the amount and virulence of the infecting agent, but enough is known to indicate plainly the existence of individual susceptibility. In an epidemic of typhoid fever, due to an infected public water-supply, where it is fair to suppose that the specific microbe is distributed with some degree of uniformity through considerable bodies of water, it is well established that not all water-drinkers, even in the same household or family, contract the disease. Such differences in the degree of individual susceptibility can hardly be referred to any deep-seated metabolic unlikeness. There are, in fact, noteworthy fluctuations in predisposition in one and the same individual. The influence of apparently slight factors, such as a change in the weather or some degree of fatigue, is sufficient to turn the scale and transform a condition of resistance into one of susceptibility. In laboratory animals used for experimental work individual variations in resistance also occur, but are not so well marked as in man, and apparently oscillate within narrower limits.

The causes of natural individual immunity are unquestionably various, some of the factors involved being more or less under control, and therefore influenced by the observances of personal hygiene, while others are dependent on qualities so fundamental that they are hardly likely to be altered during the lifetime of the individual.

Instances of the close adaptation of particular parasites to particular hosts are common throughout the whole animal and plant kingdoms, and should not be confounded with the phenomena of natural individual immunity. A close mutual relation, for example, seems to subsist between the leprosy bacillus and the human organism, and it is as little enlightening to refer the non-infectibility of the dog or the rabbit with the leprosy bacillus to "immunity" as it would be to declare that Indian corn and the tobacco plant are "immune" to Tilletia tritici, the parasite of wheat smut. The relatively few instances among bacteria of abject dependence of a single species of parasite upon a single species of host is a fact doubtless connected with the wide range of bacterial conditions of

life. It is evident that the exemption of certain races or species of animals from the attacks of certain species of parasites is not necessarily referable to the same cause or set of causes that bring about the varying degrees of resistance evinced by individuals of the same species. The problems of natural individual immunity are closely connected in some ways with those of acquired immunity.

Acquired Immunity

Acquired immunity may be either active or passive.

Active immunity is due to the direct participation of the host concerned, and depends upon increased antibody production. Such immunity is gained at the expense and often at the risk of the organism acquiring it. Immunity to smallpox may be obtained either by an attack of the disease due to natural exposure, or by deliberate inoculation of dried material from smallpox pustules into the nostrils, according to the common practice of variolation in England in the eighteenth century, introduced from the Orient by Lady Mary Wortley Montague, or by the now common method of vaccination with cowpox virus. In each and every case this immunity depends upon a specific reaction on the part of the cells and tissue of the individual host.

Passive immunity, on the other hand, involves no active generation of protective substances by the immunized animal. The latter is simply the recipient of antibody substances formed in the body of another animal and transferred to the individual to be protected. In the preparation of diphtheria antitoxin the horse is actively immunized by the injection of increasing doses of diphtheria toxin, and the blood of the horse comes to contain a protective substance, the so-called diphtheria antitoxin. When a child has been exposed to diphtheria, it is sometimes the custom to inject about 1000 units of diphtheria antitoxin (p. 289) into the body of the child for protective purposes. The child is then measurably assured against an attack of diphtheria, and is said to have been immunized passively, since its own tissues have in no way shared in the manufacture of the protective substance. Passive immunity may be quickly acquired, but is also much less permanent than active immunity, and tends quickly to disappear.

Active immunity may be brought about in a number of ways:

(*a*) By the incorporation into the animal body of living, fully virulent bacteria;

(*b*) By the incorporation of living bacteria of diminished virulence;

(*c*) By the incorporation of dead bacteria;

(*d*) By the incorporation of bacterial products secreted or excreted during the life of the microbes;

(*e*) By the incorporation of bacterial products arising from the disintegration of the cells after death;

(*f*) By the incorporation of certain micro-organisms or their products which are not associated in any way with the production of the specific affection.

These may be briefly illustrated.

(*a*) Immunity produced by the introduction of living and virulent bacteria is practically identical with the immunity that results from an attack of disease after natural exposure. In experimental work the varying facility with which this mode of immunization can be effected is in part dependent upon the susceptibility of the organism to the particular parasite. A very susceptible animal can be immunized in this way only with great difficulty or not at all. Barber[1] has shown that a single anthrax bacillus can initiate a fatal infection in a white mouse. The successful use of living cultures involves the administration first of small non-fatal doses which are increased as rapidly as possible, as indicated by the intensity of the reaction produced. The relative insusceptibility to infection by some particular channel has been also taken advantage of, as in Ferran's method, now superseded, for protective vaccination of man against Asiatic cholera. In this disease natural infection occurs by way of the alimentary tract, while the subcutaneous injection of living virulent cholera spirilla is followed by a local reaction and some fever, but by no general infection or serious consequences. Up to the present this is the only instance in which virulent cultures have been used in the immunization of man, unless, indeed, the old practice of variolation advocated by Lady Montague is to be reckoned here. This is thought to be the principle of the empiric immunization of animals against cattle-plague[2] (Rinderpest).

(*b*) Bacteria may be attenuated, that is, have their virulence diminished, in a variety of ways: (1) by growth at temperatures

[1] Barber: Jour. Infect. Dis., 1909, 6, p. 634.

[2] Bile taken from animals dying of cattle plague is used for immunization; the parasite concerned is unknown.

above the optimum, this being the usual manner of preparation of anthrax vaccines (p. 275); (2) by growth in the presence of weak antiseptics (*e. g.*, the anthrax bacillus in a medium containing carbolic acid 1 : 600); (3) by passage through the body of an animal of a different species, it being shown, for example, by Pasteur that the organism of swine plague, when passed through the bodies of rabbits, gained in virulence for rabbits but lost in virulence for swine. Pathogenic organisms may also be attenuated by growth on ordinary culture-media (pneumococcus), by continued cultivation in the presence of oxygen (bacillus of chicken cholera), by desiccation (virus of hydrophobia), and in other ways.

The injection of attenuated cultures may be sometimes followed by injection of fully virulent cultures, as in vaccination against anthrax, more effective protection being secured in this way than by the use of attenuated cultures alone. The classic example of immunization by attenuated cultures is afforded by the ordinary procedure of vaccination against smallpox. Although the specific parasite has not yet been isolated, there is no doubt that the cowpox virus, the vaccine, contains an attentuated form of the smallpox parasite, weakened in virulence by passage through the body of the heifer.

(*c*) Immunization with dead bacteria has the merit of avoiding all danger of infection and at the same time of introducing into the body the substances most intimately connected with the bacterial cell and its activities. In experimental work upon animals this method has found wide application. Vaccination of man against three important diseases—Asiatic cholera, typhoid fever, and plague—has likewise been carried out successfully with dead cultures. The particular methods used and results obtained are described elsewhere (pp. 346, 368, 489). The use of dead bacterial cells for immunization has been most advantageous in such infections as those named above, in which no powerful soluble toxin is secreted by the cell in cultures, since the toxic elements seem to be bound firmly to the cell-substance. When a high degree of immunity is sought after, as in some kinds of experimental work, the injection of dead bacteria may be followed by that of living attenuated cultures, and finally by that of fully virulent ones.

(*d*) The preparation of diphtheria antitoxin furnishes the best studied instance of immunization by bacterial products. Briefly speaking, the method consists in injecting a horse subcutaneously with small quantities of broth in which a toxin-producing strain of B. diphtheriæ has been growing, gradually increasing the doses. The horse becomes immunized by this treatment, and is able, in a few weeks, to withstand many times the dose that would have been fatal if given in the first place. This immunity is due to, or at least usually accompanied by, the accumulation of the specific antibody, the diphtheria antitoxin, in the blood of the horse. It is evident that the broth in which the diphtheria bacillus has been cultivated, usually for about one week, must contain a variety of substances secreted or excreted by the living bacterial cell, but the substance to which the immunization is usually attributed is the specific diphtheria toxin. It has been found that it is not essential that the toxin used for immunization be in a toxic condition. Toxin which has been properly detoxicated by moderate heating (toxoid) or by treatment with formaldehyde (anatoxin) or other suitable reagents will incite antitoxin production.

Antibodies may be developed by feeding an animal with specific poisons as well as by injection. Ehrlich immunized mice against ricin and abrin by feeding these animals with gradually increasing quantities of the poison until they became able to resist several hundred times the lethal dose. Some degree of immunity has been achieved by feeding animals also with bacterial toxins or with dead bacteria, but the results obtained in this way are as a rule less rapid and satisfactory than those reached by injection.

(*e*) The use of disintegrated products of the bacterial cell in immunization cannot be readily separated in practice from the two methods just considered. The use of dead bacteria must entail always the presence of some substances derived from the breaking up of the cells, and the use of broth in which bacteria have grown also involves the introduction of substances originating from dead as well as from living bacteria. At the same time some investigators have advocated the employment of material obtained by the self-digestion of bacterial cultures (autolysis), and have considered that, owing to the speedier absorption of the physiologically active substances, more satisfactory results were secured by this method.

Successful results have also been reported by Macfadyen,[1] who, following the work of Buchner on the extraction of enzymes from yeast-cells by pressure, triturated washed agar cultures of the typhoid bacillus at the temperature of liquid air, $-180°$ to $-190°$ C. At this temperature the cells are brittle and disintegrate readily without admixture with sand or other triturating substances. The resulting cell-juices were found to be highly toxic and to possess strong immunizing properties.

(*f*) Some degree of immunity toward specific infections may be developed by the use of certain kinds of bacteria or bacterial products entirely foreign to the infection in question. In this category, for example, is the undoubted protection conferred against anthrax by the injection of B. prodigiosus or B. pyocyaneus or their products. Similar instances are the use of yeast in certain pyogenic affections, and, perhaps, the retarding effect of streptococcus infection upon certain kinds of tumors. The increased resistance of the organism so treated is sometimes ascribed to the "antagonism" of the bacteria or their products, but the phenomenon may be ascribed with greater plausibility to the increased leukocytosis resulting from the injection of protein substances. (See p. 172.)

It is not always possible in practice to separate sharply the modes of immunization previously outlined. The injection of an animal with a bacterial culture entails simultaneous injection with living bacterial cells, dead cells, secretion products, and products of disintegration, and it is evident that the results obtained may be due to the concurrent action of several factors. As already pointed out, however, the methods in ordinary use involve the predominance of one or another constituent.

A combination of passive and active immunization has been found advantageous in certain cases, a potent protective serum being used to pave the way for the introduction of living virulent cultures or powerful toxins. The injection of protective sera along with the more dangerous excitants of active immunity has been used with more or less success in swine erysipelas, cattle plague, foot-and-mouth disease, and anthrax.

[1] Macfadyen: Centralbl. f. Bact., 1901, 30, p. 753; 1903, Abt. 1, Orig., 34, pp. 618, 765.

The Mechanism of Immunity

(a) **The Antitoxins.**—It was first shown by Behring and Kitasato[1] in 1890 that the immunity of rabbits and mice which had been artificially immunized against tetanus was associated with the ability of the cell-free blood to render harmless the toxic substances produced by the tetanus bacillus. The same investigators bestowed the name antitoxin upon that substance in the serum which thus nullified the toxin. The action of the antitoxin is manifested in the following direct way: If a fatal or many times fatal dose of toxin be mixed with an appropriate quantity of antitoxic serum in a flask, the injection of the mixture into a susceptible animal is not only not fatal, but wholly without injurious effect; the action of the toxin is wholly suspended because of its mixture with the serum taken from the immunized animal. This phenomenon does not depend upon the total destruction of the toxin by the antitoxin, as is shown by heating the mixture to a point sufficient to destroy the antitoxin, when it is found that the toxin remains after the heating and is able to exert its toxic power.[2] In other words, a more or less loose chemical combination of antitoxin and toxin seems to take place in the mixture, the poisonous properties of the toxin being held in abeyance as long as the union exists. The rate of reaction between toxin and antitoxin, like other chemical reactions, is dependent upon temperature, concentration, character of the medium in which the reaction occurs, and similar factors. The avidity of an antitoxin for its corresponding toxin differs in different cases; the union between tetanus toxin and antitoxin, for example, taking place less rapidly than that between diphtheria toxin and antitoxin.

The precise character of the toxin-antitoxin reaction has been the theme of much discussion. According to Ehrlich's conception the reaction is purely chemical and is essentially similar to the neutralization of a strong acid by as trong base. But precise study of the rate of toxin-antitoxin neutralization necessitated the further assumption that in a toxic filtrate there exist several substances of different toxic and combining powers, toxons, toxoids, epitoxoids, etc. This assumption of modification of the structure of the toxic molecule led to further hypotheses of great com-

[1] Behring and Kitasato: Deut. med. Wochenschr., 1890, 16, p. 1113.

[2] The method of destroying the antitoxin by heat is applicable only in certain cases, *e. g.*, pyocyaneus antitoxin; in many cases the antitoxin resists heat better than the toxin.

plexity. Ehrlich's explanation is based on the view that toxin and antitoxin behave in mixture like a strong acid and a strong base, chemical change ceasing only when there has been a practically complete neutralization of one by the other. If now, on the contrary, it be assumed that conditions are those that arise when a weak acid is added to a weak base (Arrhenius and Madsen) chemical reaction does not continue until the reagents are completely used up, but stops when an equilibrium is reached at which there are still present definite amounts of the original substance as well as of the reaction products.

A third view (Bordet, Landsteiner) looks upon toxin-antitoxin combinations of varying degrees of toxicity as due to differences in completion of saturation of the individual toxic units. The process may be compared to certain staining reactions, such as the action of iodin upon starch, a dilute iodin solution producing a light blue tinge, a stronger solution a deep blue. The coloring matter in this and other staining reactions does not stain some of the material strongly and others not at all, but distributes itself with considerable uniformity over the whole mass of stainable substance. This adsorption theory would view the action of antitoxin upon toxin as a sort of progressive attenuation, proportional to the amount of antitoxin added. Broadly speaking, the theory of Ehrlich considers the toxin-antitoxin reaction to be similar to the reaction between a strong acid and a strong base; that of Arrhenius and Madsen to the reaction between a weak acid and a weak base, and that of Bordet as an adsorption phenomenon between two colloids of opposite electric charge.

Little is clearly known about the chemical character of the antitoxins. In general, they are, like the toxins, unstable, complex bodies, readily destroyed by relatively low temperatures (65° to 75° C.), and losing strength steadily under exposure to light and air. They are very sensitive to the action of acids. They are best preserved for standards (Ehrlich) by evaporation of the sera to dryness in a vacuum at low temperature, and subsequent storage *in vacuo* in the dark at a low temperature and with protection from dampness. Attempts to obtain the antitoxin from milk or sera in purer or more concentrated form have been numerous. Treatment of immune sera with ammonium sulfate, magnesium sulfate, and other salts has shown that the antitoxins are precipitated with

and are more closely bound to the globulins[1] than to the other protein bodies in sera.

Standardization of Antitoxins.—The strength of a given antitoxic serum, that is, its value as a neutralizing agent for the corresponding toxin, is a matter of considerable practical importance. It might be supposed that it would be a relatively simple procedure to determine the fatal dose of diphtheria toxin for a guinea-pig, for example, and then the amount of serum necessary to neutralize this, thus arriving at the antitoxin content of the serum. Unfortunately, the conditions are more complex. Ehrlich found when different diphtheria toxins were used, or when the same toxin was tested at different periods, that a unit quantity of a given serum did not neutralize an equivalent number of fatal doses. The number of fatal doses that could be rendered harmless by equal amounts of the same antitoxic serum might vary within such wide limits as 30 to 130. In other words, the combining power of a given toxin for a given antitoxin is not an accurate measure of its toxic qualities. Since, however, the combining power itself remains constant within narrow limits, it is possible to establish an arbitrary standard unit upon which the relative strength of all antitoxic sera can be based. Such a standard antitoxin was first prepared by Ehrlich, and was preserved by him with all precaution against possible deterioration. A standard antitoxic serum based on Ehrlich's arbitrary standard unit is also prepared in this country by the Hygienic Laboratory of the United States Public Health Service, and is distributed every two months to the licensed producers of commercial serum. By the use of this standard antitoxin it is possible to standardize a given toxin for use in testing later the strength of antitoxic sera. Briefly, the procedure consists in determining by animal reaction two limits (Lat., *limes*): (1) the amount of diphtheria toxin necessary to neutralize exactly the standard unit of antitoxin: this is called the L_0 dose of toxin; (2) the amount of toxin which, when mixed with one unit of standard antitoxin, is just sufficient to kill in four days a guinea-pig of approximately 250 grams weight: this is designated as the L_+ dose of toxin. When these limits are established, it is then necessary to determine the smallest amount of the serum under test which, when mixed with the L_+ dose of toxin,

[1] In some animals the antitoxins are found in the euglobulin fraction, in others in the pseudoglobulin.

will prevent the death of a guinea-pig of 250 grams weight for four full days. This amount of serum is considered to contain one unit of diphtheria antitoxin.[1]

Origin of the Antitoxins.—The appearance of a toxin-neutralizing substance in the blood of an animal injected with toxin raises a number of questions as to its source. The explanation naturally suggested itself to some investigators that the antitoxin was a "modified toxin" produced by transformation of the substance injected. It is found, however, that the quantity of antitoxin developed in an animal is often much greater than the physiologic equivalent of the toxin injected,[2] and, further, that the total antitoxin content of the body may continue to increase for some time after toxin injections have ceased. The difference between the permanence of active immunity and the evanescent character of passive immunity is also sometimes accepted as evidence against the modification theory, since, if the antitoxin arose by transformation, there would be no apparent reason why it should persist in the body longer in one case than in the other. Such facts indicate that the antitoxin is probably generated in the tissues. But it is important to remember that neither toxin nor antitoxin has been isolated in pure form and current views on the nature of antitoxin—based of necessity upon speculations—must be accepted cautiously. Thus, the difference in the persistence of actively and passively acquired antitoxic immunity may be determined by the nature of the blood protein with which the antitoxic property is associated.

Since the active share of the animal body in the production of antitoxin is unquestionable, it remains to ask whether the antitoxin is a new substance hitherto unknown to the organism producing it, or whether it is present in small quantities in the normal organism and is simply increased in amount under the stimulation of toxin injection. Observation has shown that diphtheria antitoxin is found in about 30 per cent. of normal horses (Bolton,[3] Cobbett[4])

[1] Full details for making tests of the strength of a serum are given in the Report of the Committee on Antitoxin and Immunizing Sera of the American Public Health Association, Jour. Infect. Dis., 1905, Suppl. No. 1, p. 284; and in Bull. No. 21, Hyg. Lab. U. S. Pub. Health and Mar. Hos. Serv., Wash., 1905, pp. 1–92.

[2] According to Knorr (Münch. med. Wchnschr., 1898, 45, p. 321), in the horse one diphtheria toxin unit may produce 100,000 antitoxin units.

[3] Bolton: Jour. Exper. Med., 1896, 1, p. 543.

[4] Cobbett: Lancet, 1899, 2, p. 332.

and in about 50 per cent. of children, and 83 per cent. of adults, examined (Wassermann[1]). There is other evidence to the same effect, that before any toxic injection has been made or any recognizable attack of the specific disease has occurred, antitoxin and other antibodies may exist preformed in certain normal individuals. So far as has been discovered, the antitoxic substances present in the body of the normal organism are identical with those found in actively immunized animals.

As already stated, antitoxins are found in the serum of immunized animals. They may also occur, though usually in much smaller amount, in the milk. There is no reason to suppose that the antitoxins are produced in the serum, although they are found there in considerable abundance. Every physiologic consideration points to the body-cells as the place of origin of the antitoxins. Kraus and Lipschütz[2] have, in fact, shown that the extract of normal organs is richer in antitoxin against certain bacteria than is the serum of the same animal. The precise tissues or groups of cells concerned in antitoxin production have not been certainly identified, and it is not likely that they are the same in all cases. There is some evidence, however, that in dogs the spleen is able to fix a part of the antigen, and is probably the source of a considerable portion of the antibodies. Asplenic dogs do not produce hemolysins, hemagglutinins, or hemopsonins as rapidly or in as high concentration as normal dogs.[3] There is evidence that the liver is the seat of formation of certain antibodies (hemolysins, precipitins). The blood itself seems at first to take no part in the fixation of antigens, and the antibodies it contains are contributed to it by the tissues, especially the blood-forming organs such as the spleen, lymph-glands, and bone-marrow. From the foregoing statements it follows that when the antitoxins are once formed they do not reside in the body permanently, but are continually leaving it in the various excretions and secretions. This fact explains the difference in the permanence of active and passive immunity; in the latter the antitoxin is excreted from the body like other foreign substances, whereas in active immunity the supply of antitoxin is maintained above the normal level, probably through its continued manufacture by the body cells. It has been shown that when a measured quantity of

[1] Wassermann: Ztschr. f. Hyg., 1895, 19, p. 408.

[2] Kraus and Lipschütz: Ztschr. f. Hyg., 1904, 46, p. 49.

[3] Luckhardt and Becht: Amer. Jour. of Physiol., 1911, 28, p. 257.

tetanus antitoxin was injected into an animal, only one-third remained after six days and no trace could be found after twenty-one days. While the disappearance of antitoxin from the body is to be referred in part to the loss in excretions, there is evidence also that some of the antitoxin is destroyed within the body itself. This seems to be especially the case when heterologous serum (from a different species of animal) is used: the antitoxin introduced in serum obtained from an individual of the same species (homologous serum) remains longer in evidence. Thus the ordinary diphtheria antitoxin in horse serum when injected into the body of a child is eliminated more quickly than if injected into another horse. Varying results are obtained with different antitoxins and different species of animals, and the reasons for the disappearance of the antitoxin from the blood cannot be said to be understood in all cases.

Immunity and Antitoxin.—It is a pertinent question at this point how far the occurrence of antitoxin in the blood is sufficient to explain the resistance either of artificially immunized animals or of those naturally immune. The neutralization of toxin by antitoxin as demonstrated in test-tube experiments would lead to the supposition that a similar reaction takes place in the living body, and that when toxin is injected into the circulation of an immunized animal, it is rendered inert by the antitoxin in the blood in the same way as if mixed with antitoxin outside the body. In the main this is doubtless true, as shown especially by the facts of passive immunity, but there are certain phenomena that emphasize the difference between a test-tube and the living body. On grounds that will be set forth presently, there is reason to believe that the toxin has a chemical affinity for certain substances in the tissues as well as for the antitoxin in the blood. Any protective action of the antitoxin in the blood must, therefore, be due to its superior avidity for the toxin. Such superior avidity usually, but not always, exists. Some instances are known where the toxic substance unites with the tissue-substance in preference to combining with the antitoxin in the blood.[1] The affinity of the cell-substance for the toxin is not a constant quality, but fluctuates under different conditions, notably in the upper limits of active immunization. The tissues of an animal treated with increasing doses of toxin sometimes become hypersensitive to the action of the toxin and, in spite of the fact that

[1] Kraus and Lipschütz: Ztschr. f. Hyg., 1904, 46, p. 49.

large quantities of antitoxin are circulating in the blood, the toxin combines by preference with the tissue-substance and causes the death of the animal. Tissue immunity is hence not always parallel with antitoxin immunity, and the presence of antitoxin in the circulating blood cannot be the whole explanation of the resistance shown by animals immunized against toxin, although it is the most evident and often the determining factor. Among the most important antitoxins that have been produced may be mentioned: the diphtheria, tetanus, scarlet fever, pyocyaneus, symptomatic anthrax, and botulism antitoxins; the antitoxins for various bacterial hemolysins; for snake, spider, and scorpion venoms; for the toxins in the blood or secretions of the eel, salamander, and toad; and for the vegetable toxins, ricin, abrin, robin, and crotin.

The antitoxin found in the blood of some naturally immune animals may possibly be responsible in some degree for the resistance of such animals, but such an explanation is certainly not valid for many cases of natural immunity. The normal fowl exhibits almost complete immunity to the tetanus toxin, but contains no tetanus antitoxin in its blood, the toxin circulating unchanged for days after injection. The same is true of the alligator, the frog and some other cold-blooded animals (Metchnikoff). Immunity in these cases can be in no wise referable to antitoxic influence.

(b) **The Bactericidal Substances—Lysins.**—Animal blood can not only neutralize bacterial toxins, but can destroy bacteria themselves. The systematic investigation of the germicidal power of normal blood began with the work of Nuttall[1] in 1886. Nuttall showed, among other things, that the blood of one kind of animal does not have the same germicidal strength for all species of bacteria, and that one and the same species of bacterium is affected differently by the blood of different animals. Two other important facts were early brought to light: (1) that the bactericidal power is lost when the blood is heated to 56° for one-half hour (Nuttall); (2) that the cell-free serum possesses the same power as the blood itself (Buchner).[2] The germicidal power of the blood *in vitro* is often considerable, a single drop of rabbit blood being able to destroy 53,700 anthrax bacteria (Lubarsch).[3]

[1] Nuttall: Ztschr. f. Hyg., 1888, 4, p. 353.
[2] Buchner: Arch. f. Hyg., 1891, 10, p. 84.
[3] Lubarsch: Centralbl. f. Bakt., 1889, 6, p. 481.

The natural immunity of the normal organism was believed by Buchner and others to be due to the bactericidal activity of the blood, and the name *alexin* (Gr., to ward off) was suggested by Buchner for the substance that presumably exercised the protective influence.

It cannot be doubted that in certain cases the alexin content of the blood of an animal corresponds to the degree of immunity toward a particular infection. Thus the serum of the white rat, an animal that possesses a high natural resistance to anthrax, is very strongly germicidal for the anthrax bacillus. Especially significant is the increase in the germicidal power of the blood which is observed in animals artificially immunized.

The serum of guinea-pigs immunized against V. metchnikovii is strongly germicidal for this organism, while that of unimmunized guinea-pigs is devoid of specific bactericidal quality. In other cases, however, there is no relation between the resistance of an animal and the bactericidal power of its serum. An animal may be vaccinated against streptococcus infection, and indeed acquire a high degree of immunity toward streptococci, without coming to possess any specific germicidal quality in its blood when tested under extravascular conditions. Normal human serum, when tested *in vitro*, is strongly bactericidal for the typhoid bacillus, and yet this does not prevent the multiplication of this organism in the blood during an attack of the disease.

Bacteriolysis.—The relations between induced immunity and the appearance of bactericidal substances in the blood are best shown in certain experimental infections of animals, notably those caused by the cholera spirillum and the typhoid bacillus. It is elsewhere pointed out (p. 489) that a specific choleraic poison or soluble toxin is not readily demonstrable in cultures of the cholera spirillum in ordinary culture-media. In correspondence with this it is found that animals immunized against the cholera spirillum contain no antitoxin in their blood. Immunity in such cases is associated with the antagonism of the body-fluids to the living cholera spirillum rather than with any toxin-neutralizing power, as shown by a series of convincing experiments by Pfeiffer, Wassermann, and others. A guinea-pig immunized against many times the fatal dose of living cholera vibrios is no more resistant than a normal guinea-pig to a fatal dose of dead vibrios. The resistance to infec-

tion with the living microbe is correlated with an increase in the specific bactericidal power of the serum of the protected animal. Thus, normal goat serum has only slight germicidal power for the cholera vibrio, 0.02 to 0.05 c.c. being needed to protect a guinea-pig against 2 mg. of a virulent culture, while 0.0001 c.c. of the serum of an immunized goat will protect against the same dose.

The Pfeiffer Phenomenon.—The fate of cholera spirilla introduced into the peritoneal cavity of an immunized animal was first followed microscopically by Pfeiffer, who gave a detailed description of the process (Pfeiffer's phenomenon).[1] The vibrios first lose their motility, then swell up and crumble into small fragments. These fragments finally melt away and disappear, the process being likened to the dissolving of wax candles in hot water. This lytic (Gr., to loose, dissolve) action of the immune serum is manifested not only within the peritoneal cavity of an immunized animal, but also when the peritoneal fluid or blood-serum is removed from the body and brought immediately in contact with the bacteria in a test-tube.

Many experiments have since been made with bactericidal serum *in vitro*, and the course of events is found to be essentially identical with that in the body. As in the case of the antitoxic sera, the antibacterial sera are specific: a serum that is highly lytic for the cholera vibrio may be without lytic effect on the typhoid bacillus.

Hemolysis.—An important extension of our knowledge concerning sera has been effected by a study of the fate of red blood-corpuscles introduced into the animal body. The injection of the blood of a mammal or bird, for example, into the body of an animal of a different species is always followed by the appearance of a hemolyzing property in the blood of the latter (Bordet[2]). This property is specific, that is, it causes the dissolution or exosmosis of the hemoglobin out of the red corpuscles of the species from which the injected blood was derived and is without, or nearly without, action upon the corpuscles of other animals, although sometimes slightly affecting the blood of closely allied species. Thus the serum of a guinea-pig inoculated with rabbit's blood becomes hemolytic for the red corpuscles of the rabbit.

[1] Pfeiffer and Issaeff: Ztschr. f. Hyg., 1894, 17, p. 355.
[2] Bordet: Ann. de l'Inst. Past., 1898, 12, p. 688.

Not only red blood-corpuscles, but other cells are capable of giving rise to antagonistic substances, or antibodies, when introduced into the animal body. A great variety of cell-dissolving (cytolytic) sera have been produced in this way. Injection of spermatozoa leads to the appearance, in the serum of the inoculated animal, of a spermatoxic substance that first renders the corresponding spermatozoa motionless and then kills them. It has been shown that such a serum resembles a hemolytic serum in all essential features. The claim has been made that such sera are histogenetically specific, that the cells of the kidney, for example, produce a "nephrotoxic" serum, those of the liver "hepatotoxic" serum, and so on; but specificity of this kind has not been demonstrated. It is more probable that a single type of cell can provoke the formation of several antibodies which affect cells of different morphology but of somewhat similar chemical constitution.

The bacteriolytic and the hemolytic sera, therefore, fall under the same general head, and the common cytolytic phenomena exhibited by the two kinds of sera are strikingly alike. It must be emphasized at this point that the lytic antibodies produced against bacteria, red blood corpuscles or other antigens are merely special cases of a general biological phenomenon. Whenever protein-containing substances (antigens) gain entrance to the blood stream, antibodies are engendered. If the antigen is a formed body capable of dissolution, the lytic nature of the antibody is demonstrable. If the antigen is a pure substance (*i. e.*, egg albumin) in true or colloidal solution, lytic properties of the antibody are not demonstrable, and only agglutinating or precipitating properties (see p. 185) may be evidenced. A large part of our knowledge of the cytolytic sera and antibodies in general is due to the genius of Paul Ehrlich[1] and of Jules Bordet.[2]

The bactericidal sera possess the following important characteristics. When heated to 55° C. for one-half hour, the bactericidal power is lost; such sera are said to be inactivated. The addition of a small quantity of normal serum to heated serum, however,

[1] Ehrlich, Paul: "Gesammelte Arbeiten zur Immunitätsforschung," Berlin, 1904. [English edition: "Studies in Immunity," transl. by Charles Bolduan, New York, 2nd ed., 1910.]

[2] Bordet: "Studies in Immunity," collected and translated by Frederick P. Gay, New York, 1909.

restores the original potency. The relation between normal sera and immune sera may be expressed in tabular form as follows:

	Germicidal Power
Normal serum	+
Immune serum	+++
Heated immune serum	±
Normal serum + heated immune serum	+++
Heated normal serum + heated immune serum	±

There is, therefore, no escape from the conclusion that the bactericidal property is due to the action of two substances or properties, one that is present in the normal serum and in unheated immune serum, and is identical with Buchner's alexin; and a second substance of superior heat-resistance which is present in small quantities in normal serum and appears in much greater amount in the serum of immunized animals as a consequence of repeated inoculation with specific germs. This second substance is the so-called *sensitizing substance* of Bordet or the *amboceptor* of Ehrlich.

An important factor in the production of antibacterial sera is the virulence of the culture employed. The use of highly virulent cultures conduces to the development of a particularly potent serum, and in experimental work it has been found desirable to use freshly isolated cultures of high virulence or cultures whose virulence has been exalted by animal passage. As a preliminary to the use of such hypervirulent cultures attenuated cultures may be employed.

The important question whether the bactericidal factor in normal serum (alexin) is identical with that in the blood of immunized animals is answered in the affirmative by most investigators, although there are a few dissenting voices. Both bacteriolytic normal and immune sera behave alike toward temperature and other influences, can be reactivated by unheated normal serum, and possess the same complex constitution. The lytic power of normal serum, like that of immune serum, appears to be due to the combined activity of two substances.

Differences between Antitoxic and Antibacterial Sera.—The fundamental distinction between the antitoxic and the bacteriolytic sera has been set forth in the preceding pages. The antitoxic sera act directly upon the poison secreted by the living bacterial cell and neutralize its toxic property, while the bacteriolytic sera affect the bacteria themselves and destroy them or paralyze their action. Since the antibacterial sera are without effect upon the

formed toxin, they are mainly useful in practice as a means of protecting against bacterial invasion, while the antitoxic sera (*e. g.*, diphtheria) may be employed to combat an infection already in progress or to prevent an infection in a person who has been exposed by contact with an incipient or active case of disease. Broadly speaking, the antitoxic sera are curative although sometimes used prophylactically, the antibacterial sera protective, although some antibacterial sera (*i. e.*, antipneumococcus) are used therapeutically. It must not be inferred that a given serum cannot be both antitoxic and bactericidal. If a horse be injected with a diphtheria culture containing both diphtheria bacilli and diphtheria toxin, the resulting serum will be not only antitoxic, but to some extent bacteriolytic.

The bacteriolytic sera have not yet been applied very successfully to the treatment of disease in man. This has been a stumbling-block to the advance of serum-therapy because most of the antisera hitherto produced are of this type. One reason for the failure of the bacteriolytic sera in human therapeutics may be that a serum of this sort produced in the body of one of the lower animals does not find suitable conditions for its specific action in the body of another species, such as man. There are facts which seem to support this view. The relative degree of success attained by vaccination with protective sera in several diseases is mentioned in connection with special topics. (See especially Asiatic Cholera, p. 489, and Typhoid Fever, p. 346.)

(c) **The Phagocytes.**—The brilliant researches of Metchnikoff[1] have definitely established the active share in combating bacterial invasion taken by certain white blood-corpuscles, denominated phagocytes, or "devouring cells." Metchnikoff's views have been sometimes referred to as the phagocyte "theory," but certain facts regarding the protection afforded by phagocytes are indubitably demonstrated.

A significant distinction exists between a local reaction and a general septicemic infection. The bacilli of chicken cholera injected subcutaneously into a rabbit produce no local inflammation, but multiply rapidly throughout the body, and the animal soon dies; the same bacilli injected into a guinea-pig provoke a strong local inflammation, the bacilli remain localized at the point of intro-

[1] Metchnikoff: "Immunity," Cambridge, 1905.

duction, and spontaneous recovery takes place. In general, the degree of resistance shown by the organism is measured by the intensity of the local inflammatory reaction.

Perhaps the most characteristic feature of the local reaction or inflammation is the gathering of the leukocytes at the affected point, and this has been shown by Metchnikoff to rest on a broad biologic basis. In certain lower forms of animal life, such as the ameba, food-particles are engulfed bodily and digested within the cell. In multicellular animals, like sponges and jelly-fish, intracellular digestion by ameboid cells also plays an important rôle, and even in the higher metazoa both free-moving and sessile cells retain the power of devouring foreign particles. In the course of the metamorphosis of invertebrates—like the echinoderms and the insects—and of vertebrates—such as the frog—it has been shown that the superfluous tissues are picked apart by the leukocytes bit by bit and carried to another part of the organism. The absorption of larval organs by the activity of the phagocytes is closely connected with the behavior of the phagocytes toward foreign substances introduced into the body. Carmine granules, for example, are readily ingested by the leukocytes of warm-blooded animals as well as by the ameba. The resorption of the catgut used in surgical operations is a familiar instance of the digestion of foreign substances in the human body, and, like the disappearance of larval organs, is attributed to phagocytic activity. In specific cases of atrophy and absorption much discussion has arisen concerning the relative share of the phagocytes and the body-fluids in bringing about the changes observed, some investigators maintaining that the solvent action of the body-fluids is sufficient to account for the destruction of useless or alien material in the organism. While it may be sometimes difficult to assign to this latter factor its true value, there can be no question that Metchnikoff and others have conclusively established the fact that the phagocytes can and do intervene in some of the typical processes of absorption and larval metamorphosis.

According to Metchnikoff, the phagocytes, besides acting as digesting cells and as scavengers, are also the chief defenders of the vertebrate organism against invading bacteria. "The diapedesis of the white corpuscles, their migration through the vessel wall into the cavities and tissues, is one of the principal means of defense possessed by an animal. As soon as the infective agents have

penetrated into the body, a whole army of white corpuscles proceeds toward the menaced spot, there entering into a struggle with the micro-organisms"[1] (Fig. 24).

Dog serum, for example, is without bactericidal power upon anthrax bacilli. If, however, anthrax bacilli are injected into a dog, they are quickly taken up by the leukocytes. The most plausible interpretation of these facts would seem to be that the natural immunity of the dog towards anthrax is due to the destruction of the bacilli by phagocytosis. In other instances where a normal animal is susceptible to infection with a particular microbe no

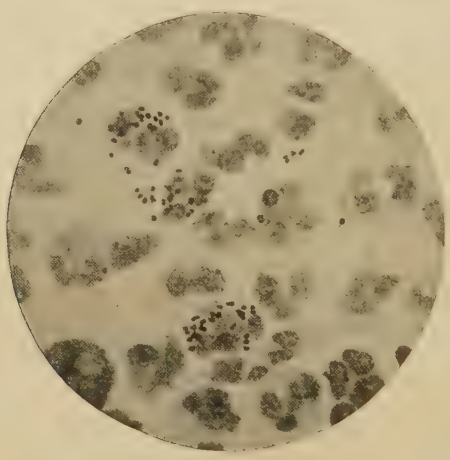

Fig. 24.—Phagocytosis of gonococci by leukocytes (Hicks).

phagocytosis is observed, but after the animal has been immunized by the injection of living or dead bacteria or their products, the phagocytes of the immune animal actively destroy the specific bacteria, no corresponding increase in the bactericidal power of the serum being observed.

It was recognized by Metchnikoff that, in addition to the free phagocytes (microphages) or polymorphonuclear leukocytes which comprise 72 to 75 per cent. of the white blood-cells, there are also certain larger cells (macrophages), many of them fixed, which possess phagocytic properties. The endothelial cells of the peritoneum and the giant cells of the bone-marrow as well as the mononuclear leukocytes belong to the class of macrophages. The microphages appear

[1] Metchnikoff: "Immunity," Cambridge, 1905, p. 548.

to be specially concerned with the acute infections, the macrophages with those of more chronic type, such as tuberculosis.

Phagocytosis may be considered as consisting of three stages: first, the bringing together of bacteria and leukocytes; second, the taking up or engulfing of the bacteria by the leukocytes, and finally, the digestion of the bacteria within the leukocytes. Apparently the main if not the only resistance offered by bacteria to this process is connected with the first stage. Certain bacteria appear to produce chemical substances which have the power of repelling phagocytic approach (negative chemotaxis). Bordet showed that virulent streptococci cause phagocytes to move away from their neighborhood, while avirulent streptococci attract phagocytes. The amount of the substances present in or produced by the host (opsonins, tropins) whose presence renders bacteria attractive to leukocytes may be increased in various ways. (See below.)

In a number of instances natural immunity appears to be due to activity of the phagocytes.

Opsonins.—Phagocytosis may be intensified or diminished in various ways. Several early observers noticed that the power of leukocytes to destroy micro-organisms is greatly increased by the addition of serum from immunized animals, and through the investigations of Wright[1] in England, Hektoen[2] in the United States, Neufeld[3] in Germany, and their associates, an explanation of this interesting phenomenon has been obtained. These investigators have demonstrated that the activity of the phagocytes is conditioned by the presence in the blood and other fluids of certain substances which act in some unknown manner upon bacteria and prepare them for digestion by the phagocytes. These bacteriotropic substances have been termed *opsonins* (Gr. ὀψωνέω, I cater for, prepare food for). Opsonins are present to some extent in the blood of normal animals, but they can be materially increased in amount by immunization.

It is a simple matter to demonstrate the existence of opsonins in a given serum by mixing the serum with bacteria suspended in physiologic salt solution. Leukocytes washed free from serum will take up some, but not a great many, of the bacteria. If, how-

[1] Wright: Proc. Roy. Soc., 1903, 72, p. 357; 1904, 73, p. 128 *et seq.*

[2] Hektoen: Jour. Amer. Med. Assoc., 1906, 46, p. 1407.

[3] Neufeld: Deut. med. Wochenschr., 1904, 30, p. 1458; Ztschr. f. Hyg., 1905, 51, p. 283; Centralbl. f. Bakt., 1906, I, Ref., 37, p. 763.

ever, the bacteria are first treated with opsonic serum, they will be taken up in much larger numbers by the washed leukocytes. The opsonin enters into a more or less close union with the bacteria, as shown by the fact that opsonized bacteria can be washed in salt solution and still remain sensitive to phagocytosis. By these and other experiments it has been fully demonstrated that a substance is present in immune sera, and to a less degree in normal sera, which can so change or sensitize bacteria as to render them more liable to phagocytic destruction.

Opsonic Technic.[1]—In determining the amount of opsonin in a given serum the following technic is employed:

It is necessary to have (1) blood-serum from the sick person and from a healthy person or persons; (2) leukocytes; (3) a suspension of the organism, the opsonin for which is to be measured. A sterile 1 per cent. sodium citrate solution in normal salt solution is placed in an ordinary glass centrifuge tube; a sufficient amount of blood, *i.e.* a few drops, is allowed to drop into this solution from a puncture of the finger or lobe of the ear; this tube is then centrifuged until the leukocytes appear as a white cream over the sediment of red blood-corpuscles. The solution is then drawn off by means of a pipet, and the tube is filled with normal salt solution, the contents well mixed, and centrifuged again.

The blood from which serum is to be obtained is gathered into little U-shaped tubes made from glass tubing with a diameter of 3 mm., the lobe of the ear being pricked by means of a small lancet, and the blood allowed to run into the U-tube by capillary attraction; the tubes may be marked with blue pencil for identification; the tubes are centrifuged until the serum is separated as a clear layer above the corpuscles. (These tubes may be sealed with wax if necessary for transportation.)

The bacterial suspensions, except that of the tubercle bacillus, which must be prepared by special methods, are made from twenty-four-hour-old cultures in broth or on solid media; if solid media are used the fluid of condensation is removed and the growth washed off by means of salt solution. Suspensions of the desired density are obtained by adding normal salt solution; clumps should be

[1] The description of technic follows the method employed in the John McCormick Memorial Institute for Infectious Diseases, and has been kindly furnished me by Professor Hektoen.

broken up either by blowing into the suspension through a pipet or by centrifugation.

In one of two watch glasses is placed the leukocytic cream which is drawn off from the centrifuge tube by means of a capillary pipet; some of the bacterial suspension is placed in the second glass; the capillary end of a right-angled capillary pipet is gently thrust into the serum in the U-tube, and the serum allowed to enter to a marked point; a small amount of air is now drawn into the capillary end and the tube is dipped into the leukocytic cream—previously carefully mixed—and the leukocytes drawn up to the same height as the serum; another air bubble is aspirated into the tube, and the bacterial suspension drawn up to the marked point.

The same process is carried out with the patient's serum as well as with normal serum obtained either from one person or from a mixture of the sera from several persons. The elements in the tubes are carefully mixed by drawing up the contents into the elbow of the tube and gently blowing the mixture back and forth. The tubes are then placed in an ordinary incubator at 37° C., and allowed to remain there for about fifteen minutes, when smears are made in the following way: The mixture is blown out on a glass slide after having been gently drawn back and forth several times, and a little square of cigarette paper is placed behind the drop of blood, which is then drawn toward the other end of the slide, thus forming a smear. The smears are dried in the air and may be stained with carbol thionin or other suitable stain.

The bacteria in at least 50 leukocytes should be counted, 25 from each edge of the smear. If the counts are uneven, more cells should be counted, and always the same number in the control as in the smear with the patient's serum. Clumps of leukocytes should not be counted.

The suspensions of bacteria and of leukocytes should be of such density that on the slide made from the mixture with normal serum there should not be more than 5 bacteria per leukocyte.

The opsonin may be estimated also by the dilution method; specimens are prepared in the usual way, except that sera, normal and immune, are diluted by means of normal salt solution or Ringer's solution. One mixture is made without serum, salt solution or Ringer's solution being used in its place, in order to determine the amount of spontaneous phagocytosis. The dilution of serum which

gives the same amount of phagocytosis as a mixture without serum is considered as the point of opsonic extension.

The *opsonic index* is a mode of expressing the relative amount of opsonins in a serum when compared with the normal standard. It is obtained by dividing the average number of bacteria taken up by a leukocyte under the influence of a given serum, by the average number taken up by a leukocyte under the influence of standard normal serum and under otherwise perfectly comparable conditions. Thus:

	Average Number of Bacteria per Leukocyte (50 Counted)
Serum of tuberculous patient + washed leukocytes + suspension of tubercle bacilli	3
Serum of normal individual + washed leukocytes + suspension of tubercle bacilli	4
Salt solution + washed leukocytes + suspension of tubercle bacilli (control)	0.1

The opsonic index of the tuberculous patient in this illustration would be 3 ÷ 4, or 0.75.

The technic of opsonic work seems peculiarly liable to lead to different results in the hands of different observers,[1] and there can be little doubt that much experience and great care are needed to obtain uniform and comparable results.

Certain facts regarding opsonins have, however, been practically established. Opsonins for many different bacteria are present in the sera of most, if not all, animals. Investigators are now agreed that after the injection of a suitable dose of dead bacteria there is usually a fall in the opsonic index of the injected animal, the so-called negative phase, and that this is followed by a rise above normal, and then by a more or less gradual return to normal. Natural infections in many cases are accompanied by a similar change in the opsonic index. It has been found in pneumonia, for instance, by independent observers that in the early stages of pneumonia the pneumococco-opsonic index is below normal, and that at about the stage of crisis the index rises considerably above the normal, returning again to normal in the uncomplicated cases leading to recovery. A similar course has been noticed in the

[1] See, for example, Bull. Johns Hopkins Hospital, 1907, 18, pp. 232–255; Jour. Amer. Med. Assoc., 1907, 49, p. 1249; R. E. Walker, Jour. Med. Res., 1908, 19, p. 237.

streptococco-opsonic index in scarlet fever, the diphthero-opsonic index in diphtheria, etc.

Opsonins are largely destroyed by heating to from 54°–60° C. for thirty minutes; this seems to be due to the destruction of a substance which favors the opsonic action of a heat-resistant factor; it is the latter which is specifically increased in immunization. In this respect and some others opsonins resemble other complex antibodies. They are, however, probably distinct from lytic amboceptors and agglutinins, as shown by the facts that a normal serum may be lytic but not opsonic, and, *vice versâ*, that immunization may give rise to one of these antibodies and not to others, and that the opsonic, agglutinating and lytic effects of serum are not destroyed in equal degree by heat. There is good reason to hold that opsonins are specific to the same extent as other antibodies.

The opsonic method of treatment has been used especially by Wright and his associates. The aim of the method is to maintain the opsonic index, and consequently the phagocytic power of the blood, by the use of "properly adjusted and interspaced doses" at a high level. Frequent determinations of the opsonic index are considered by some to be necessary in this mode of treatment in order to guard against too great and too frequent production of the negative phase. Others would attach less importance to the opsonic content as an index to the administration of killed bacteria, and more importance to the clinical conditions and symptoms. The inoculation of dead bacteria and bacterial products has given distinctly encouraging results in the hands of Wright and others, particularly in the treatment of infection with pyogenic cocci, and of forms of tuberculosis localized elsewhere than in the lungs.

It has been shown by a number of investigators, especially by Hektoen[1] and his associates, that the phagocytic power or activity of the leukocytes is subject to considerable variation independently of variation in the opsonic content of the blood. This inherent phagocytic power of the leukocytes varies with respect to certain bacteria at least, even in persons in apparently perfect health. In the child at birth the leukocytes are somewhat less active phagocytically than in the adult; they grow less active for a few months, and then more active, the adult standard for streptococci, pneumococci, and staphylococci being reached about the third year (Tunnicliff).

[1] Hektoen: Jour. Amer. Med. Assoc., 1911, 57, p. 1579.

The phagocytic power of leukocytes has been found to be greater than normal for certain bacteria in pneumonia, scarlet fever, and other conditions in which there is acute leukocytosis, when the outlook is favorable. The increase in activity in such cases may be due to the predominance of young leukocytes.

In view of all these facts, there can be no doubt that a local inflammatory reaction or a general increase in the number of leukocytes (leukocytosis) is a process of distinct advantage to the organism. The injection of certain substances which increase the general leukocytosis (collargol, nucleic acid) has been practised by Mikulicz[1] and others with a considerable degree of success in attempts to enhance the resisting power of the body, prior to abdominal operations that involve grave danger of infection. The production of leukocytosis, however, is in itself of little value unless at the same time the amount of opsonin in the blood, the specific opsonic index, is high enough to favor phagocytosis. The simultaneous stimulation of leukocytosis and production of opsonins would, therefore, seem to be the object to be aimed at in many of the bacterial infections that have so far proved most refractory to serum therapy.[2]

(d) **Ehrlich's Receptor Theory.**—The existence of toxin-neutralizing and bactericidal substances in the body fluid of immunized animals has already been set forth. However these facts are interpreted, it must be remembered that the facts themselves are securely established. An ingenious and fruitful attempt to explain the mode of origin and manner of action of antitoxic and bactericidal sera was made by Paul Ehrlich[3] in his widely known side-chain or receptor theory. It is well to bear in mind that the much-used standard diagrams are pictures of the imagination and not representations of established fact. Whatever be the ultimate fate of the speculations that have been built up around the central hypothesis, there can be no doubt that knowledge of immunity and the immunizing processes was greatly increased by the stimulus given to research through the doctrine of receptors.

The receptor theory starts with the assumption that the various cells of the animal body, having to obtain their nutriment from the

[1] Mikulicz: Archiv. f. klin. Chirurg., 1904, 73, p. 347.

[2] An excellent résumé of the history of research on opsonins is given by Hektoen in the Middleton-Goldsmith lecture, Jour. Amer. Med. Assoc., 1906, 46, p. 1407.

[3] Ehrlich: "Studies on Immunity," New York, 1906.

blood or lymph with which they are bathed, are endowed with the power of extracting from the ambient fluid those substances necessary to their life and well-being. This power of food appropriation is definitely localized in certain cell-substances, the so-called cell-receptors, which have combining affinities for food-substances. These food receptors have been conceived as standing in the same relation to the main body of the cell that the side-chains of certain complex chemical molecules of known composition hold to the central molecular nucleus. The receptors may be of simple consitution, adapted to the taking up of relatively simple substances, or they may be very complex and able to anchor large and complex proteid molecules of various kinds. Each cell may contain a large number of receptors of different affinities and degrees of complexity.

It is a plausible conception that when bacteria or other alien cells or their products are introduced into the body, the combining affinities of certain receptors may be satisfied by bacterial substances just as by the similarly constituted food molecules. The anchoring of toxic substances, however, unlike that of food-substances, is followed by damage to the cell and loss of the particular side-chain or receptor that unites with the toxic element. When injury to the main body (Leistungskern) of the cell is not carried too far, repair can take place and the receptors be regenerated. Following a principle enunciated by Weigert respecting regeneration of tissue cells, there is a tendency, in such cases of regeneration of lost parts, toward overcompensation, receptors being formed in excess of the needs of the cell and the surplus being discharged into the blood-stream. *These free receptors are the antibodies*, the antitoxins, agglutinins, antibacterial substances, and the like. The diphtheria antitoxin in the blood, then, is the same substance which, when in the cells, may be a peril to the cell by virtue of its affinity for the diphtheria toxin. If the toxin did not find in the body any substance with which it could combine,—for which, in other words, its haptophore group (p. 113) possessed an affinity,—it would be wholly inert and impotent as far as that organism was concerned. When, however, the free receptors are brought in contact with the toxin either inside or outside of the animal body, they unite with the haptophore group of the toxin molecule, thereby preventing the latter from entering into combination with the cell-receptors and perhaps damaging the cell irreparably.

There is evidence that the various bacterial toxins become bound in each case to particular cells of the organism. The tetanus toxin when mixed *in vitro* with emulsions of fresh organs manifests an affinity for different organs in different animals. In man, the horse, and the guinea-pig only the central nervous system is able to bind the tetanus toxin. This is altogether in accord with the clinical and histologic characteristics of tetanus. If a mixture of tetanus toxin and guinea-pig brain emulsion in suitable proportions is injected into a susceptible animal, the animal is entirely unaffected, just as if tetanus antitoxin (free receptors) had been used in place of fresh cell-substance (cell-receptors). The toxin is firmly bound in both cases and is quite unable to exert its toxic effect. That a real combination occurs between the toxin and the brain-substance is further shown by mixing tetanus toxin and normal guinea-pig brain emulsion and allowing them to remain in contact for a certain period; on centrifugalizing the mixture the supernatant fluid is found to be entirely toxin-free. In some other animals, for example, the rabbit, other organs besides the central nervous system are capable of binding the tetanus toxin.

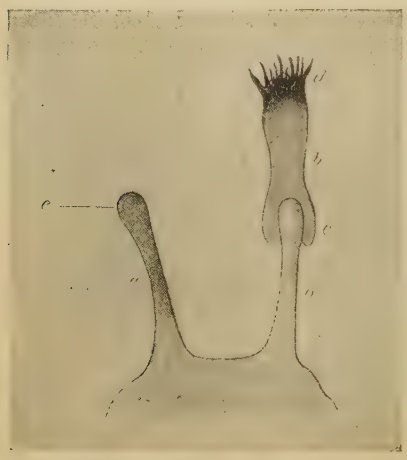

Fig. 25.—Graphic representation of receptors of the first order and of toxin uniting with the cell-receptor: *a*, Cell-receptor; *b*, toxin molecule; *c*, haptophore of toxin molecule; *d*, toxophore of toxin molecule; *e*, haptophore of the cell-receptor (Ehrlich).

Metchnikoff has shown that the alligator injected with tetanus toxin does not sicken, and that this is because of the lack of sensitiveness of its central nerve-cells to the toxophore group; experiments *in vitro* prove that certain organs of the alligator are able to bind the tetanus toxin, and, furthermore, in accord with this, tetanus antitoxin is produced by injection of tetanus toxin into the living alligator. It follows that antitoxin production does not necessarily depend upon the susceptibility of a given animal to a given toxin. It is sufficient that the animal possesses receptors able to bind the haptophore group of the toxin. So far as antitoxin formation is

concerned, it would seem to be a matter of indifference whether or not the toxophore portion of the toxin molecule is present to injure the cell.

The constitution of bactericidal sera is more complex than that of antitoxic sera, as already pointed out, and the mode of action of the former is correspondingly less simple. Ehrlich has postulated the existence in the living cell of three kinds of receptors, designated respectively as of the first, second, and third order (Figs. 25, 26, 27).

The receptors of the first order are the simplest, being those which unite with the toxin, and, if regenerated in excess, appear in the blood-stream as antitoxin (Fig. 25, *a*). The receptors of the second order are supposed to be more complex and adapted to the assimilation of relatively complex protein molecules (Fig. 26, *c*). The agglutinins are considered to be receptors of this nature. The agglutinins are quite resistant to heat, 70 to 80 degrees, as a rule, being needed to effect their disappearance, and, once removed from the serum, no reactivation can be effected by the addition of fresh normal serum. In this respect the purely agglutinative serum differs from the unheated sera containing receptors of the third order.

Fig. 26.—Graphic representation of receptors of the second order and of some substance uniting with one of them: *c*, Cell-receptor of the second order; *d*, toxophore or zymophorous group of the receptor; *e*, haptophore of the receptor; *f*, food-substance or product of bacterial disintegration uniting with the haptophore of the cell-receptor (Ehrlich).

In the case of the bactericidal sera the specific antibody (receptor of the third order) possesses two affinities, one for the specific bacterial cell, one for the labile, activating substance present in normal serum (Fig. 27). The peculiar function of the antibody lies in bringing together in close relation this ingredient of normal serum and the bacterial cell; hence it has been called the intermediary body (Zwischenkörper), but in more recent nomenclature it is

known, in agreement with Ehrlich's conception of its nature, as the *amboceptor*. The constituent of normal serum is designated by Ehrlich and his followers as the *complement*. Every amboceptor is consequently possessed of two different combining groups: the complementophile, having an affinity for the complement, and the cytophile, having an affinity for some specific cell. A number of different amboceptors may coexist in the body of the same animal; the blood of one animal may be bactericidal for a variety of different microbes, and the specific amboceptor for one kind may be removed by appropriate methods without affecting those of other affinities.

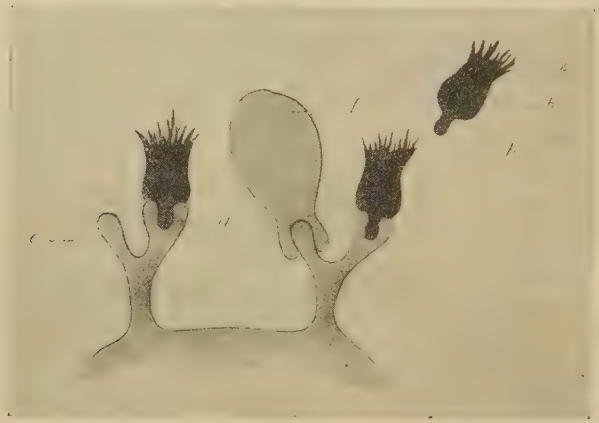

Fig. 27.—Graphic representation of receptors of the third order, and of some substance uniting with one of them: *c*, Cell-receptor of the third order, amboceptor; *e*, one of the haptophores of the amboceptor with which some food substance or product of bacterial disintegration, *f*, may unite; *g*, the other haptophore of the amboceptor with which complement may unite; *k*, complement; *h*, the haptophore, and *z*, the zymotoxic group of the complement (Ehrlich).

The complement, on the other hand, is not specific and is probably a substance common to the serum of the higher animals.

Since it is assumed to be the active agent in the various cytolytic sera, the question arises as to the nature of this substance, and here it must be admitted that little or nothing is known beyond the bare physical characteristics already stated. At one time the complements were supposed to be of an enzyme nature, but there is little to support this view.

Along the line of Ehrlich's receptor theory, Welch[1] has introduced the conception of the throwing-off of antibodies by patho-

[1] Welch: Bull. Johns Hopkins Hosp., 1902, 13, p. 285.

genic micro-organisms within the body of their host. Just as the cells of the host generate substances antagonistic to the cells of invading parasites and their products, so it is supposed that the parasites, by a similar mechanism, may produce amboceptors with affinities for certain tissue-cells. Linked with a suitable complement such amboceptors may exert a poisonous action upon the cells of the host.

(e) **The Neisser-Wechsberg Phenomenon.**—A singular phenomenon, studied especially by Neisser and Wechsberg,[1] is the reaction obtained when varying amounts of immune serum are added to constant volumes of normal serum and bacterial emulsion. When very small amounts of immune serum (amboceptor) are added, no bacteriolysis takes place; on increasing the amount of immune serum complete bacteriolysis finally occurs; but on adding still greater quantities of immune serum, bacteriolysis once more fails to appear. From these and similar experiments it was concluded by these investigators that a "*deviation of complement*" occurs under conditions where the amboceptors are in great excess, that is to say, the complement unites with the unbound amboceptors rather than with the amboceptors that have become attached to the bacterial cells. Hence with a limited amount of complement some of it is deviated away from the amboceptor-antigen complex to the free amboceptor and is consequently unable to cause bactericidal action. There is considerable doubt whether this is a correct interpretation of the Neisser-Wechsberg phenomenon. Indeed, it is generally agreed to-day that of all the conceivable interpretations, this is the least likely because there is no single experimental observation in harmony with an assumption that amboceptor and complement combine directly. The phenomenon is more simply explained as due to the presence in excess of one of the reagents in a colloidal mixture and to the interfering effects commonly caused by such an excess in colloid reactions.

(f) **Complement Fixation or the Bordet-Gengou Phenomenon.**—This is a reaction of great practical and theoretical interest. The nature of the reaction may be illustrated as follows:

(A) Complement (fresh guinea-pig serum) + typhoid bacilli + inactivated normal rabbit serum.

(B) Complement (fresh guinea-pig serum) + inactivated normal rabbit serum.

[1] Neisser and Wechsberg: Münch. med. Wchnschr., 1901, 48, p. 697.

(C) Typhoid bacilli + inactivated normal rabbit serum.

(D) Complement (fresh guinea-pig serum) + typhoid bacilli + inactivated antityphoid rabbit serum.

(E) Complement (fresh guinea-pig serum) + inactivated antityphoid rabbit serum.

(F) Typhoid bacilli + inactivated antityphoid rabbit serum.

If these mixtures are incubated for a few hours and then rabbit corpuscles that have been "sensitized" with inactivated serum specifically hemolytic for rabbit corpuscles added to each mixture hemolysis will occur in all except (C) and (F), which contain no complement, and (D) in which the specifically sensitized bacilli have absorbed the complement and so prevented its subsequent action on the sensitized erythrocytes added. That is, the mixture of complement, antigen (typhoid bacilli), and specific antibody (alexin in the immune typhoid serum) is no longer capable of producing hemolysis. This is interpreted as meaning that the bacilli when specifically sensitized absorb or fix the complement and thus prevent its action on the sensitized erythrocytes subsequently added.

The complement fixation reaction is used extensively in the diagnosis of several infectious diseases, notably of syphilis, in the "Wassermann reaction." (For details of technic, see p. 514.) It is also used to some extent for the identification of bacterial species. In such mixtures as those described, if the specific antibody is present, the complement is fixed *and no hemolysis occurs when sensitized red corpuscles are added;* if the specific antibody is absent, the complement is of course free to complete the hemolytic system, and hemolysis will result.

It is now generally agreed that Ehrlich's qualitative receptor analysis of immunity and immune reactions has outlived its usefulness. From the presentation which has been given in the preceding pages, it must be apparent that it is only an ingenious application to immune processes of an hypothesis on cell nutrition and injury that has long since been discarded by students of physiology or pathology. Ehrlich's hypotheses have crumbled under the weight of the special assumptions which were necessitated by each new discovery. Recent investigators have found it more profitable to discard Ehrlich's hypotheses in favor of conceptions borrowed from colloid chemistry. As rapidly as the dynamics of colloid systems are elucidated and become familiar to students of infectious disease, they are being applied profitably to the study of immunity.

It is not yet timely to formulate a general theory on these grounds. Hence, Ehrlich's pictures are still retained, although their values are doubtful.[1]

Other Reactions Produced by Bacteria and Other Antigens

When bacterial cells and their products are injected into an animal, the cells of the animal react in such a way as to give rise to a variety of substances besides the antitoxins and bacteriolysins (amboceptors) already considered. Among the better known and most important of these are the agglutinins.

(a) **The Agglutinins.**—If the blood or blood-serum of an animal previously inoculated with the typhoid bacillus is added to a suspension of typhoid bacilli, the latter soon becomes motionless, and after a while the individual bacilli become aggregated in irregular masses. The same process of clumping usually occurs if, instead of the blood of an injected animal, the blood of a typhoid fever patient be employed. The reaction is specific, although not absolutely so. That is, the serum of either a typhoid patient or an animal injected with typhoid bacilli will agglutinate typhoid bacilli in high dilutions, while other bacilli, as a rule, are unaffected, although it is true that those more closely related to the typhoid bacillus, such as B. coli and B. paratyphosus, may be slightly clumped, especially by the lower dilutions. Many other bacteria as well as those of the colon-typhoid group are agglutinated in a similar manner by their respective antisera.

The agglutination of bacteria by blood-serum has been utilized in two ways: first, in the diagnosis of certain specific diseases like typhoid fever, where the serum from the suspected case is added in appropriate dilutions to a suspension of typhoid bacilli; and, second, in identifying bacteria, for which purpose a serum of known agglutinating properties is mixed with a suspension of the germ whose character is to be determined. The former, the Gruber-Widal test, has come to be extensively used in the diagnosis of typhoid fever, and when carefully controlled, is a valuable aid in diagnosis; the identification of bacteria by the agglutinative reaction is, on the other hand, less satisfactory, and there are many objections to its unqualified acceptance.

[1] For a trenchant criticism of the side chain theory see Manwaring: Jour. Immunol., 1926, 12, p. 177.

Technic.—The technic of the agglutination test calls for the observance of manifold precautions. An agar culture eighteen to twenty-four hours old is preferably employed. From this a faintly turbid suspension in sterile physiologic salt solution is prepared. The suspension of the bacteria must be homogeneous; for some cultures a thorough shaking will suffice, for others recourse must be had to passage through filter-paper. In any case a uniform method of preparation must be employed in every series of experiments, and care taken to obtain as nearly as possible the same number of bacteria in a given volume of the suspension. The nature and reaction of the medium on which the bacteria are grown, the age of the strain and its origin, and other factors influence the course of agglutination. Some strains are naturally more readily agglutinable than others; some may be inagglutinable even by a potent serum.

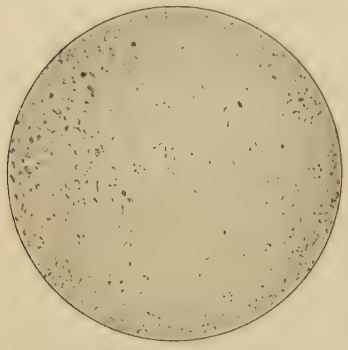

Fig. 28.—Typhoid bacilli, unagglutinated.

The serum for the test may be obtained from a blister made by the application of a cantharides plaster, or from blood drawn from the ear-lobe or finger-tip and centrifugalized or allowed to clot in a sterile tube. The substance in the serum that causes the agglutination of the bacteria is known as *agglutinin*. It is fairly permanent and may persist in dried blood or serum for a long time in unchanged strength. In public health work a few drops of blood may be dried on a strip of aluminum foil, filter paper or other substance which is then mailed to a central laboratory, where flakes or extracts of the dried blood are weighed and dissolved in appropriate amounts of salt solution so that the test is made as accurately as with fresh blood.[1]

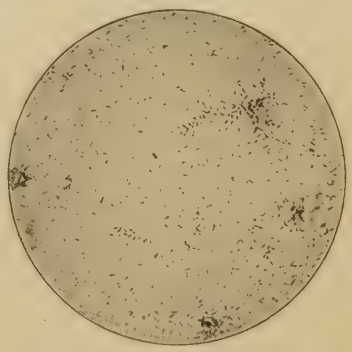

Fig. 29.—Typhoid bacilli partially agglutinated.

[1] See Wesbrook: Jour. Infect. Dis., Suppl. No. 1, 1905, p. 315.

Appropriate dilutions of the serum or blood with physiologic salt solution are mixed with measured quantities of the bacterial suspension, and the process of agglutination followed either with the naked eye or with the microscope. The microscopic examination, made with high powers, shows a gradual cessation of motility in such organisms as the typhoid bacillus, accompanied by the sticking together first of a few cells, then of larger numbers, until in typical and decisive reactions large masses of agglutinated cells are found which are plainly visible with a low power. The maximum clumping is generally reached in about six to eight hours (Figs. 28, 29, 30). Sometimes "spontaneous agglutination" occurs, so that a suspension to which no serum has been added should always be observed along with the others, in order to avoid this source of error.

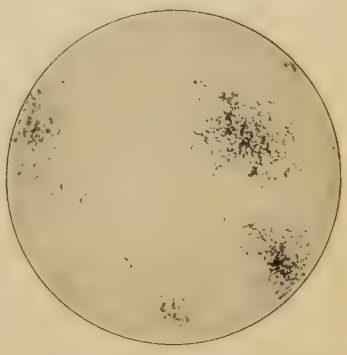

Fig. 30.—Typhoid bacilli, showing typical clumping by typhoid serum.

The naked-eye test gives, as a rule, more trustworthy results than the microscopic. Varying dilutions of serum are mixed with a definite quantity of bacterial suspension in small thin-walled glass tubes, and placed in the incubator. The tube containing only the bacterial suspension remains cloudy, while those tubes to which agglutinating serum is added show a general clearing-up of the fluid, ranging from the appearance of a flaky deposit on the walls of the tube, with high dilutions of serum, to a complete sedimentation of the bacteria and clear supernatant fluid.

A convenient procedure is as follows: Small quantities of the serum are diluted with sterile salt solution (0.85 per cent. NaCl) so as to effect the desired dilutions (for example, 1 : 50, 1 : 100, 1 : 500, 1 : 1000). Filtered suspensions in salt solution of a twenty-four-hour agar culture of the organism tested should be used. To obtain the dilution desired, mixtures of the serum dilution and the suspension may then be made. The tubes are incubated at 37° C., usually for two hours, and then placed in the refrigerator for sedimentation. When complete agglutination takes place bacteria collect in clumps at the bottom, forming a flocculent sediment and leaving the super-

natant fluid clear. Control tubes of the bacterial suspension without serum and with normal serum should be carried in each series.

Temperatures above 60° C. diminish the agglutinability of bacteria; agglutinin also is weakened by heating to from 60° to 70°, and is destroyed at 75°. Serum dried and protected from light retains its power indefinitely.

Properties and Mode of Action of Agglutinins.—Agglutination is brought about by the chemical interaction of two substances, one a constituent of the agglutinating serum, the *agglutinin*, the other a bacterial substance, the *agglutinogen*. The latter substance receives its name from the fact that when introduced into the animal body it stimulates the formation of agglutinin. In terms of Ehrlich's receptor theory the agglutinogen contains a haptophore or combining group, which enables it to unite with certain receptors of the tissue cells. These are said to be "receptors of the second order" (Fig. 26), and to contain a haptophore group which binds them to the agglutinogens, and a zymophore group of enzyme-like activity which brings about the change in bacterial cells that leads to their agglutination. The phenomena of agglutination, however, are so complex that no relatively simple structural hypothesis fits the facts. The most plausible explanation is a colloidal reaction, due to a process of molecular adhesion.

It is essential that some mineral salt be present in order that agglutination shall occur. Following out this clue, considerable light has been shed on the real nature of the agglutinating process. It is found that the same laws that govern the precipitation of colloids in suspension and finely divided particles, like kaolin, also hold for the agglutination of bacteria. The ability of a salt to agglutinate bacteria and to precipitate colloids depends on the degree of dissociation and the valency, speed of migration, and decomposition tension of the kation. In a word, agglutinin is supposed to reduce the amount of negative electricity with which bacteria are charged and so render the bacteria more susceptible to the precipitating action of the salts.[1]

There is reason to believe that specific agglutination occurs in two phases: first, the union of antibody and antigen, that is the sensitization of the specific bacteria by the agglutinating serum;

[1] An excellent summary of the physico-chemical conception of agglutination has been given by Buchanan (R. E. Buchanan: Jour. of Bacteriol., 1919, 4, p. 73).

second, the flocculation of the sensitized bacteria by the electrolytes present in the fluid. It has been suggested that the first phase consists in a specific coating of the bacteria by the globulin of the antibody. The two reactions may of course go on simultaneously. Whatever the explanation, the reason for the specific character of the union between organism and antibody remains quite unknown, that is, the reason why the globulin of antityphoid serum should coat the typhoid bacillus but not the dysentery bacillus. In the second phase the bacteria, by virtue of their coating, behave like particles of denatured protein and flocculate when their charge is reduced to a critical level.

Not bacteria only, but other free cells, are clumped both by normal and immune sera. The red blood-cells of any species of animal are agglutinated by the serum of an animal which has been previously injected with cells of similar origin. The "hemagglutinins," so far as they have been studied, resemble the bacterial agglutinins in all essential features. Some bacterial cultures have been found to contain hemagglutinins, and it is possible that the thrombi observed in the blood-vessels after death from certain infectious diseases may be due to hemagglutinins of bacterial origin.

Substances that agglutinate bacteria and erythrocytes are found in the blood of many normal animals. The serum of a young uninoculated horse, for example, has been observed to agglutinate paradysentery bacilli in a dilution of 1:1000 (Park).[1] The relation between normal and immune agglutinins is not known.

Group Agglutination.—The serum of an animal inoculated with a given micro-organism agglutinates not only the particular species used for inoculation, but very often also other organisms biologically related to the infecting agent. As a rule, agglutination of the organism used for inoculation, the so-called "homologous" organism, is the most marked, that is, it will occur with higher dilutions of serum than is the case with other organisms of the same group. Thus the serum of a rabbit inoculated with typhoid bacilli shows a higher agglutinating power for the typhoid bacillus than for other members of the colon-typhoid group, although the agglutinating value of the serum for B. enteritidis, B. coli, or other members of the group may be distinctly greater than that of normal serum.

[1] Park: Jour. Infect. Dis., Suppl. No. 2, 1906, p. 1.

Sometimes, however, the accumulation of "group agglutinins" and "specific agglutinins" seems to follow a very irregular course. This is especially true in the serum of an animal like the horse, which contains a high proportion of normal agglutinins, so that group agglutinins may be even more abundant than specific agglutinins after repeated injections. Park has observed one instance where the serum of a horse after eighteen injections with the "Manila" strain of the paradysentery bacillus agglutinated a colon bacillus more strongly than it did the homologous organism.

The *absorption or saturation test of Castellani* (1902) based on the discovery of absorption of agglutinins by Bordet (1899) has proved of practical value in eliminating the source of error due to group agglutination. It was shown by Castellani that the serum of an animal immunized against a certain micro-organism, when saturated with that micro-organism, loses both its agglutinating power for that organism and also for all the other varieties it originally acted upon. Thus immune serum A which agglutinates the homologous organism A and the related organisms B and C (group agglutination) will lose its agglutinating power for all these organisms when absorbed by a suspension of organism A. If, however, it be absorbed by organism B it will lose all its agglutinating power for B, but it will agglutinate A as strongly as before. The absorption test has been widely used in studies on the typhoid-paratyphoid group and on meningococci. Its value in differentiation within certain other groups is still in question. The technic of the absorption test is important. The size of the minimum absorbing dose necessary to remove all the agglutinins must be carefully determined. In the final test the dose of the homologous organism should be somewhat but not greatly in excess of the minimum. In all absorption tests little weight should be attached to slight differences in agglutination.

The application of this test may be shown by an example. The undifferentiated organism X is agglutinated by the serum of animals immunized to A and B.

Organism.	Immune Serum A Titer.[1]	Immune Serum B Titer.	Immune Serum X Titer.
A	5000	3000	5000
B	3000	5000	5000
X	3000	3000	5000

[1] The "titer" is the highest dilution of the serum which causes complete or nearly complete agglutination of the bacteria.

Organism.	Immune Serum A Absorbed by A	Immune Serum A Absorbed by B
A	0	5000
B	0	0
X	0	0

Organism.	Immune Serum X Absorbed by A	Immune Serum X Absorbed by B
A	0	3000
B	5000	0
X	5000	0

It is evident that so far as agglutination reactions are a criterion of relationship, organism X belongs with B rather than with A, although the ordinary agglutination tests with unabsorbed serum did not make this apparent.

The Relation between Agglutinating and Bactericidal Power.—Although some observers have maintained that there is a direct relation between the agglutinating and bactericidal properties of a serum, the following facts show that bacteriolysins and agglutinins may be entirely distinct: (*a*) The bactericidal power of a serum is destroyed at 56°, while the agglutinins resist a temperature of 62° or higher. (*b*) In the serum of an animal injected on successive dates with a bacterial culture the respective increases in the bactericidal and agglutinating power do not run a parallel course or indeed show any connection. (*c*) The agglutinin may be absorbed from a serum, leaving the bactericidal power of the serum unimpaired.

(*b*) **The Precipitins.**—When the germ-free filtrates from broth cultures of bacteria are mixed with the respective antisera produced by animal inoculation the formation of a powdery precipitate occurs.[1] The precipitation thus produced is approximately specific in its nature, the filtrate from a typhoid culture giving a precipitate with typhoid immune serum, but not, for example, with cholera immune serum. The substance in the immune serum that provokes precipitation has been termed *precipitin.*

Bacterial precipitins are by no means the only kind that may be produced by this method. A great variety of albuminous bodies when injected into animals give rise to corresponding antibodies which possess the power of causing precipitation of the substance used for inoculation. Injection with milk brings about the forma-

[1] Kraus: Wiener klin. Wchnschr., 1897, 10, p. 736.

tion of a precipitin which throws down the casein of the milk used for injection, but does not act on the casein of the milk of other animals. Egg-albumin likewise gives rise to precipitins that are specific. Indeed any antigen may arouse the production of precipitating antibodies, whether or not other antibodies (lytic, agglutinative, etc.) are demonstrable.

A particularly important development in the study of the precipitins has been the utilization of the specific character of the reaction for the purpose of medicolegal investigation.[1] The serum of an animal which has been injected with human blood produces a precipitate when mixed with human blood even in high dilutions but has no such effect upon the blood of the lower animals. A simple, delicate, and highly trustworthy method for distinguishing human blood-stains is thus afforded, and the value of the precipitation test in skilled hands has been abundantly demonstrated.

The rabbit is the most suitable animal for the preparation of precipitating antisera. The initial dose should be small, about 2 c.c., for example, in the case of an animal serum, and this may be followed at intervals of five to seven days by gradually increasing doses—*e. g.*, 3, 5, 8 c.c.—up to six or seven times the original amount. About one week after the last injection the animal is bled, the serum collected and filtered through a Berkefeld filter, to insure perfect clarity, and sealed in small brown glass tubes, without addition of any preservative, until needed. For the best results a precipitating antiserum should be of high potency. According to Uhlenhuth, who has had extensive experience with the test in forensic medicine, such a serum should have the following titer: "Upon addition of 0.1 c.c. of the serum to 2 c.c. of the corresponding blood solutions diluted 1 : 1000, 1 : 10,000, and 1 : 20,000, the reaction should appear almost instantaneously in the thousandth, within three minutes in the ten-thousandth, and within five minutes in the twenty-thousandth dilution; turbidity is observed at the bottom of the small test-tube, which should not be shaken after the serum has been added." With such a high-potency serum very dilute solutions of the protein to be examined must be prepared. Solutions in normal saline solution corresponding approximately to a thousandfold dilution of the protein give satisfactory results.

[1] Hektoen: Jour. Amer. Med. Assoc., 1918, 70, p. 1273.

By the use of this method remarkable results have been obtained by Uhlenhuth[1] and others.

"A butcher, accused of robbing and murdering three persons, stated that certain blood-stains found on his shirt sleeves were referable to his having slaughtered a calf. By the biological method, however, the human origin of the blood was proved. This result was an important link in the chain of circumstantial evidence which was so convincing that the accused was condemned to death. Shortly before his execution he made a full confession.

"In a poaching case, one of the accused persons, who was also suspected of being a receiver of stolen goods, asserted that blood-stains found on his meat-chopping board were not due to deer's blood, but to that of wild ducks. By the biological method, however, we determined the presence of deer's blood, besides that of ducks, thus proving the guilt of the accused."

One of Uhlenhuth's interesting discoveries is the observation that antiserum obtained from closely allied species affords the best means for determining the differentiation of certain organisms. The serum of monkeys inoculated with human blood gives a marked turbidity with solutions of human blood, while not reacting at all with monkey's blood. Such a separation is difficult if not impossible when an antiserum from the rabbit is used. In the same way the antiserum from a rabbit inoculated with fowl's blood gives a precipitate with both fowl's blood and pigeon's blood, but if pigeons be inoculated with fowl's blood the resulting serum precipitates fowl's blood and does not react at all with pigeon's blood.

By this method also it has been found that Anopheles mosquitoes feed not only upon man, but also upon cattle and pigs.

The substance that reacts with the precipitin ("precipitinogen") seems to persist for a long time (at least sixty-six years) in dried blood; mixture with other bloods does not invalidate the reaction. While the blood precipitins are highly specific, they may produce a slight reaction with the sera of animals biologically related. The phenomenon of blood precipitation has been utilized by Nuttall and others for the purpose of throwing light upon the biologic affinities of forms of animal life.[2] Nuttall has established the interesting fact that the precipitin produced by human blood will throw down a more abundant precipitate from the blood of

[1] Uhlenhuth: "Praktische Anleitung zur Ausführung des biologischen Eiweissdifferenzierungsverfahrens mit besondere Berücksichtigung der forensichen Blut- und Fleischuntersuchung," Jena, Gustav Fischer, 1909.

[2] The subject of precipitation is treated with great clearness and fulness in a monograph by Nuttall, "Blood Immunity and Blood Relationship," Cambridge, England, 1904.

the old-world monkeys than from that of the South American species.

The precipitation test has also been applied to the differentiation and identification of meats. By its aid horse meat, for example, may be distinguished from beef. It may be used in the identification of any antigenic substance, especially of any complete protein.

The Mechanism of Agglutination and Precipitation.—The phenomena of precipitation and agglutination are essentially the same. Recently some significant advances have been made toward an elucidation of the mechanisms involved. It appears that the antigen in either case is, or is very similar to, a particle in the colloidal state and bears an electro-negative charge (*i. e.*, is electro-negative by comparison to the fluid in which it is suspended). The normal stability of the antigen solution or suspension probably depends upon the balance of three types of forces: those associated with the Brownian movement, which serve to overcome gravitational sedimentation; surface tension of the fluid on the surface of the particles which tends to bring the individual particles together; and the mutual repulsion of the particles, because of their common electro-negative charge, which functions to counterbalance the cohesive force of surface tension.[1] Upon the introduction of any substance which increases the cohesive forces or sufficiently reduces the electrical charges there follows agglomeration, flocculation and precipitation of the antigen. Reduction of the electrical charge is probably of greater significance than increase of the cohesive forces. On proteins[2] and on bacteria,[3] the electrical charges are commonly greatest in distilled water near neutrality. The addition of salts, acids, or alkalies reduces the charge, and if sufficient is added precipitation and agglutination occur. The charges appear to have their origin in conditions (the Donnan equilibrium) which are dependent upon the semi-permeability of the colloidal or bacterial surface, and in the case of bacteria appear to be independent of the viability of the organisms. This is in harmony with the well-known observation that a suspension of dead bacteria will serve as well as live bac-

[1] It is not entirely certain, although recent studies have rendered it probable, that these are only surface tension forces.

[2] Loeb, J.: "Proteins and the Theory of Colloidal Behavior," New York, 1922.

[3] Northrop and DeKruif: Jour. of Gen. Physiol., 1921–22, 4, p. 639; Winslow, Falk and Caulfeild: Ibid., 1923, 6, p. 177.

teria in an agglutination test. When an immune serum is added to a suspension of bacteria the negative charge on the cells is reduced, and if the serum is added in appropriate dilution the charge is brought within the agglutination zone. If too little serum is added the charge is insufficiently reduced, and if too much is added the charge is inverted (the bacteria become electro-positive) and no agglutination occurs in either case. The stabilizing effect of an excess of serum (normal or immune) which inverts the charge probably accounts for the so-called pro-agglutinoid zone. Electrolytes (acids, alkalies, and neutral salts), like sera, modify the charges on colloids or bacteria, and their effects in a precipitation or agglutination reaction are probably directly cumulative with those of the immune body. The rôle of NaCl in agglutination tests is probably of such an order. The discovery that the precipitation reaction—like the agglutination reaction—is dependent upon the hydrogen ion concentration[1] and is delimited by P_H values which approximate the points at which the antigen begins to lose its charge, makes it seem highly probable that precipitin, like agglutinin, either is or simulates an electro-positive substance. It is significant that entirely similar substances are present in normal sera.[2] The more specific identity of these bodies and, indeed, the identity of agglutinin and precipitin are still entirely uncertain.

(*c*) **Specific Soluble Substances—Residue Antigens.**—It was found by Zinsser and his associates[3] that certain non-protein constituents of bacterial cells are definitely specific and have an immunological value. These remarkable substances react with antibodies, but when injected into an animal are not themselves capable of giving rise to antibodies.

The chemistry of these soluble specific substances has been studied especially by Avery and Heidelberger.[4] The soluble specific substances of the pneumococci, for example, have been found to be of a carbohydrate nature. Each fixed type of pneumo-

[1] Mason: Bull. Johns Hopkins Hosp., 1922, 33, p. 116; Hirsh: Jour. Infect. Dis., 1923, 32, p. 439; Falk and Caulfeild: Jour. Immunology, 1923, 8, p. 239.

[2] Northrop and DeKruif: Jour. Gen. Physiol., 1921–22, 4, p. 639.

[3] Zinsser: J. Exp. Med., 1921, 34, p. 495; Zinsser and Mueller: J. Exp. Med., 1925, 41, p. 159.

[4] Avery and Heidelberger: J. Exp. Med., 1923, 38, p. 81; Ibid., 1925, 42, p. 367.

coccus (Types I, II and III) (p. 232) contains a chemically different carbohydrate (polysaccharide) which reacts with the serum that has been produced by immunizing an animal with the homologous type of pneumococcus. The antiserum of Type I precipitates solutions of the corresponding carbohydrate. As pointed out by Avery, therefore, immunization of an animal with whole intact pneumococcus cells leads to production of a specific agglutinating substance, and of a precipitating substance specific for the type. Injection of the specific polysaccharides leads to no antibody production. Injection of the isolated free cell protein gives rise to protein antibodies (not agglutinins) that are specific for the species but not for the type. The specific soluble substance in those bacteria that have been studied is closely related to, if not identical with, the capsular substance of the cell.

Different kinds of bacteria yield soluble specific substances that may differ markedly in chemical constitution. Avery and Heidelberger have summed up the significance of their studies in these words: "If final proof be brought for the conception that the capsular zone of the organism is largely composed of the carbohydrate substances, is part of the defense mechanism of the cell and is the site of its initial contact with antibody, then these soluble bacterial polysaccharides acquire new significance not only in the serological reactions of the cell, but in the actual processes of infection and immunity in the host."

(*d*) **Anaphylaxis or Hypersensitiveness—Allergy—Protein Sensitization.**[1]—Animals injected with certain substances become sensitized or rendered hypersensitive to a subsequent injection with the same substance. The name *anaphylaxis* (Gr. against protection) has been used (Richet) for this phenomenon to explain its apparently opposite character to the protective effect of immunization. The nature of the reaction is illustrated by the "Theobald Smith phenomenon" (1904). Smith observed that guinea-pigs which had been injected with horse-serum in the course of work on the standardization of diphtheria antitoxin developed striking symptoms, frequently fatal, if they were given a second injection

[1] See summaries by H. Zinsser: Arch. Int. Med., 1915, 16, p. 223; A. F. Coca: Tice's Practice of Medicine, Prior and Co., New York, 1920; T. H. Boughton, Jour. Lab. Clin. Med., 1920, 5, p. 597; H. G. Wells: "Chemical Pathology," 4th ed., Philadelphia, 1920, p. 191; Ibid.: Physiol. Reviews, 1921, 1, p. 44.

of horse-serum some days later. The hypersensitiveness of rabbits caused by repeated injection of horse-serum was also clearly shown by the work of Arthus[1] (1903). The study of this reaction was taken up by Otto in Germany and by Rosenau and Anderson[2] in the United States. Zinsser[3] has given a clear description of the anaphylactic reaction in the guinea-pig. "If a properly sensitized guinea-pig receives a second injection of an antigen after a suitable incubation time a very characteristic train of symptoms ensues. There is usually a short preliminary period—lasting either a fraction of a minute or several minutes according to the violence of the reaction and the mode of administration—during which the pig appears normal. At the end of this time the animal will grow restless and uneasy, and will usually rub its nose with its forepaws. It may sneeze and occasionally emit short coughing sounds. At the same time an increased rapidity of respiration is noticeable and the fur will appear ruffled. In light cases the animals may remain in this condition, with further irregularity and difficulty of respiration, possible discharges of urine and feces; then gradual slow recovery may set in, with complete return to normal in from thirty minutes to several hours. In more severe cases these preliminary stages are rapidly followed by great apparent weakness. The animals fall to the side, the legs and trunk muscles twitch irregularly, and the respiration becomes slow and shallow; the thorax never entirely contracts, but remains in a more or less expanded condition. The very evident dyspnea is of an inspiratory character. The excursions of the lung itself seem to grow shallower and shallower in spite of apparent strong inspiratory efforts, the volume of the thorax and lung remaining in the expanded condition. At this stage evidences of motor irritation may appear, in that the animal may arise and attempt to run. More often, however, in this phase general convulsions set in, often several times repeated, and in these the animals usually die.

"On the other hand, after cessation of convulsions they may lie perfectly still on the side, as though paralyzed, the breathing becoming gradually slower and more shallow, finally ceasing

[1] Arthus: Compt. rend. de la Soc. Biol., 1903, 55, p. 817.

[2] M. J. Rosenau and J. F. Anderson: Bull. 29, Hyg. Lab. Mar. Hosp. Service, 1906.

[3] H. Zinsser: "Infection and Resistance," New York, 1914, p. 363.

entirely. The heart may continue to beat for a considerable time after the breathing has stopped."

A definite period must always elapse between the first and second injection in order that the toxic effect shall be manifested. In the sensitization of guinea-pigs against horse-serum this period, as a rule, is from ten to twelve days. The toxic action of the horse-serum upon the guinea-pig has been studied with especial fulness.[1] Sensitization may be effected by very small quantities; Rosenau and Anderson reported that in one instance 0.000,000,1 c.c. of horse-serum was sufficient to render a guinea-pig susceptible. Wells,[2] using pure protein, has shown that the minimum sensitizing dose of crystallized egg albumen is 0.000,000,05 gm. and the minimum fatal sensitizing dose 0.000,001 gm., the fatal reacting dose being intravenously 0.000,05 gm. and intraperitoneally 0.000,5 gm.

The liability to react hypersensitively is transmitted from mother to offspring, the young of actively sensitized female guinea-pigs being themselves hypersensitive. The male does not transmit this quality. Passive hypersensitiveness may be induced by transfer of serum from a sensitized animal to a normal one. Animals that survive the second dose of protein are *desensitized*, that is, rendered very insensitive to the specific protein. Isolated strips of smooth muscle are likewise capable of desensitization. Highly immunized animals will not give reactions, probably because of free antibody in the blood preventing the antigen from reaching the sensitized cells. This condition, which is plainly different from desensitization, is called *anti-anaphylaxis*.

The anaphylactic reaction has also been adequately studied in the dog and the rabbit, with the striking result that in each animal species the symptoms are different, but are always constant for the same species irrespective of the particular antigen used.

Among the most significant observations on anaphylactic shock is the discovery that some of the principal features of the anaphylactic condition can be demonstrated upon a strip of non-striated muscle removed from the body (Schultz, Dale). These experiments have strengthened the belief "that stimulation of non-striated muscle is an essential, and probably the essential factor of acute anaphylactic shock" (Wells), and it is tempting to

[1] Rosenau and Anderson: Bulls. 29, 30, 36, Hyg. Lab. Mar. Hosp. Service; Jour. Infect. Dis., 1908, 5, p. 85.

[2] Wells and Osborne: Jour. Infect. Dis., 1911, 8, p. 66.

suppose that differences in the distribution of non-striated muscle in the various organs account for the difference in the behavior of various animal species in which a condition of hypersensitiveness has been induced.

There seems little doubt that anaphylaxis is to be regarded as an antigen-antibody reaction and that the substances capable of behaving as antigens in this particular reaction (anaphylactogens) are all protein substances. Gelatin and some other incomplete proteins appear to be absolutely devoid of antigenic power.

The specificity features of the anaphylactic reaction are highly interesting and correspond closely to those exhibited by precipitin and complement fixation reactions. Wells and others have shown that the antigenic specificity of a protein is not determined by its biological origin, but by its chemical composition. Casein from goat milk evokes precisely the same anaphylactic response as casein from sheep milk, but chemically dissimilar proteins from the same animal body fail to show any common immunological relation.

Particular groups within the protein molecule—the amino-acids—are probably responsible for the specificity of anaphylaxis and other antigen-antibody reactions. Both chemical composition and isomeric arrangement doubtless play a part in the close correspondence that exists between antigen and antibody.

Theories of anaphylaxis are essentially of two types, the proteolytic and the physical or colloidal explanation. The former is based on the demonstration by Vaughan and others that the protein molecule can be split by various chemical methods into a toxic and a non-toxic portion. The toxic portion when injected produces in animals a condition very similar to that of anaphylactic shock. Such observations were thought to indicate that the second injection of antigen was sensitized by the specific antibody produced by the first injection, and that then the serum complement proteolyzed the antigen liberating the toxic fraction (anaphylatoxin) with its typical poisonous effects. It was found, however, that isolated muscle tissue shows an immediate response to the specific antigen, and it is difficult to reconcile this fact—the absence of an incubation period—with the hypothesis of liberation of a poison through proteolytic action. Serum complement has never been shown to be a proteolytic ferment, and attempts to demonstrate the presence of a

poisonous substance in the blood of animals in anaphylactic shock have not been successful. Unformed substances, such as kaolin, added to normal serum render the serum toxic, as also does the mere clotting of plasma. For these and other reasons the theory that anaphylaxis is due to the products of protein cleavage, supported though it is by a mass of ingenious experiments, has lost ground in the course of recent investigation. The physical or colloidal explanations, on the other hand, are still somewhat vague and indefinite, but it is certainly tempting to suppose that alterations in colloidal equilibrium within the living cell are in some way responsible for the anaphylactic phenomena. At all events there seem to be no facts directly contradicting this supposition.

Although there is a good deal of evidence to support the view that precipitins are the anaphylactic antibodies, this is not completely established. Most investigators are agreed that the site of the reaction leading to anaphylactic shock is in or on the tissue cells, and not in the blood. When the blood of a sensitized animal is injected into a normal animal, the latter does not become hypersensitive until 6–36 hours later and the development of the sensitization is parallel to the disappearance of the injected sensitizing antibody (precipitin). Hence it is supposed that the anaphylactic state is dependent upon the fixation of the antibody upon the tissue cells.

The relation of anaphylaxis to serum disease, to hay-fever, and to drug hypersensitiveness is far from clear. While acute, undoubtedly anaphylactic reactions to serum injections are sometimes observed in man, the ordinary serum sickness with its skin manifestations is not believed by some investigators to be a true anaphylactic reaction; the evidence is conflicting. Hay-fever is commonly regarded as an instance of sensitization with the pollen of various plants, and strikingly successful therapeutic results have been reported as due to apparent desensitization. Here also there is no unanimity of opinion. The numerous cases of food idiosyncrasy to egg albumin, milk, and other protein foods are well established instances of true anaphylactic reactions; a condition of desensitization may be produced by careful feeding.

The relation of anaphylactic reactions to bacterial infection is evident in many ways. Certain features of infectious diseases, such as the period of incubation and various skin reactions, resemble

anaphylactic processes. Bacterial proteins, like other proteins, have been shown to give anaphylactic reactions. The relation of anaphylaxis to immunity appears in such phenomena as immediate reaction to vaccines occurring in some protected individuals, and the effects observed by Koch when tuberculous guinea-pigs are reinjected with tubercle bacilli; in the latter case the tubercle bacilli introduced are at once disintegrated.[1]

[1] Cf. Zinsser: "Infection and Resistance," 3rd edition, New York, 1923; Wells: "The Chemical Aspects of Immunity," New York, 1925.

CHAPTER IX

THE STAPHYLOCOCCI

Genus: Staphylococcus. Parasites. Cells in groups and short chains, very rarely in packets. Generally stain by Gram. On agar streak good growth, of white or orange color. Glucose, maltose, sucrose, and often lactose fermented with formation of moderate amount of acid. Gelatin often liquefied very actively.

Type species is Staphylococcus aureus, Rosenbach.

The bacteria most commonly found in boils, abscesses, carbuncles, and similar suppurative processes in man belong to the group of staphylococci. The presence of staphylococci in pus was first shown by Pasteur[1] (1880) and later by Ogston[2] (1881). Micrococci were obtained in pure culture by Becker[3] in 1883, but their causal relation to the suppuration of wounds and to osteomyelitis was first clearly brought out by the work of Rosenbach[4] in 1884.

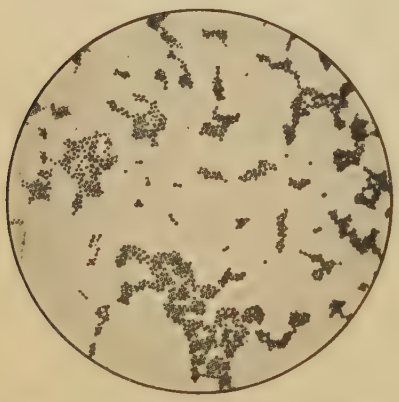

Fig. 31.—Staphylococcus aureus. Fuchsin; × 1000 (Günther).

Several races or varieties of staphylococci have been differentiated in the course of later investigation. One of these, Staphylococcus (pyogenes) aureus, is found frequently in connection with pathologic processes in man; the other varieties, although very similar, differ in slight particulars from this type. Staphylococcus (pyogenes) albus, for example, is distinguished from Staphylococcus (pyogenes) aureus by its failure to produce a golden-yellow pigment.

[1] Pasteur: Bull. de l'Acad. de Méd., 1880, 9, p. 447.

[2] Ogston: Brit. Med. Jour., 1881, 1, p. 369.

[3] Becker: Deut. med. Wchnschr., 1883, 9, p. 665.

[4] Rosenbach: "Mikroorganismen bei d. Wundinfektionskrankheiten," Wiesbaden, 1884.

Morphology and Staining.—The cells of Staphylococcus aureus are generally aggregated in loose, irregular masses which have been likened to clusters of grapes, and have given the generic name to this organism (Fig. 31). The dimensions of the individual cocci vary within rather narrow limits, the diameter of the cells ranging between 0.7 and 0.9 μ. The ordinary anilin dyes stain the cells readily; no decolorization occurs with Gram's method. In a preparation made directly from pus or from a pure culture, not only irregular clusters of cells can be observed, but also tetrads, diplococcus forms, and short chains. It is hence often difficult to determine from stained preparations whether only true staphylococci are present or whether there is an admixture of streptococci or other forms.

Physiologic Requirements. The optimum temperature is about 28° C., but growth can also take place at temperatures as high as 42° C., and as low as 8° to 9° C. The cocci thrive best in the presence of oxygen, but grow to some extent under anaërobic conditions. As regards choice of culture-medium, this organism is not so fastidious as many other pathogenic bacteria. It grows upon the ordinary laboratory media (Fig. 32), and also, although less luxuriantly, upon protein-free media containing creatin or asparagin as the source of nitrogen. The golden-yellow pigment which distinguishes the *aureus* variety from other staphylococci is formed in especial abundance upon blood-serum or upon a starchy medium, such as potato or rice. The thermal death-point is not constant, different strains appearing to vary greatly in their resistance, some succumbing only after thirty minutes' exposure to a temperature of 80° C., while others are destroyed within the same time at a much lower temperature. Considerable resistance is displayed toward drying, experiments showing a retention of vitality for many days and even months, in cultures dried upon silk threads and desiccated over calcium chlorid. Toward the

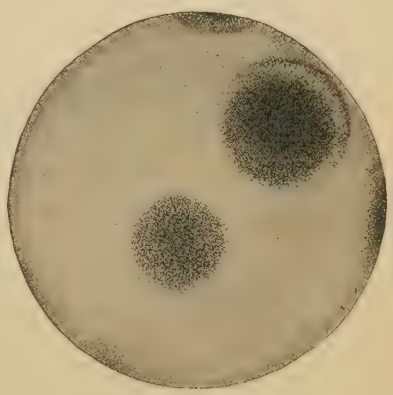

Fig. 32.—Staphylococcus aureus: colony two days old, seen upon an agar-agar plate; × 40 (Heim).

chemical substances ordinarily used as disinfectants Staphylococcus aureus also exhibits more than the average resistance. It is, indeed, one of the hardiest of the non-spore-forming bacteria.

Staphylococcus aureus is a well-nigh constant inhabitant upon the surface of the skin and also upon the various mucous surfaces of man and other animals. Apart from its occurrence in the air of hospitals, stables, and similar situations where its presence is readily explicable, it is found relatively infrequently in nature except in association with the animal body.

Products of Growth.—The golden-yellow pigment which is produced by this organism and which is probably a lipochrome, is formed most abundantly upon carbohydrate media and in the presence of free oxygen. A specific gelatin-liquefying enzyme or gelatinase is formed in gelatin and broth cultures and has been separated from the cultures by filtration (Fig. 33). Other enzymes, such as rennin and maltase, are produced under suitable conditions. Dextrose, maltose, sucrose, and glycerol are usually fermented under suitable conditions with acid but no gas production. Milk inoculated with staphylococci is coagulated by the acid resulting from the fermentation of the milk-sugar; the precipitated casein, as a rule, remains undissolved.

Fig. 33.—Staphylococcus (pyogenes) aureus, gelatin stab-culture; four days.

Certain strains when grown under suitable conditions produce a substance that acts upon the stroma of red blood-corpuscles in such a way as to cause the dissolving out of the hemoglobin. This hemolytic substance is formed both upon agar plates and in broth cultures. The hemolytic qualities of filtrates of Staphylococcus aureus have been especially studied by Neisser and Wechsberg.[1] The specific hemolysin, known as staphylolysin, is completely destroyed by heating for twenty minutes at 56° C. An antibody to staphylolysin is produced by inoculating an animal with hemolytic filtrates, and there is other evidence that staphylolysin possesses a structure analogous to that of diphtheria toxin and is endowed with a stabile haptophore and a labile toxophore group. Many observers claim that a direct relation exists between virulence and hemolytic power, but others have failed to discover any such connection.

[1] Neisser and Wechsberg: Zeitschr. f. Hyg., 1901, 36, p. 229.

A substance that kills leukocytes is also present in staphylococcus filtrates; this has been termed leukocidin, and, like staphylolysin, is a true toxin. The presence of leukocidin may be determined by an ingenious method devised by Neisser and Wechsberg, which consists in using the reduction of methylene-blue that is effected by live leukocytes as a measure of the integrity of the latter. The extent of retardation or disappearance of the reducing action measures the degree of injury wrought upon the leukocytes by the leukocidin.

From the physiologic effects which follow the injection of staphylococcus filtrates or cell-substance it is inferred that, besides the hemolysin and leukocidin, other toxic bodies are generated by these organisms, but practically nothing is known of the nature of these bodies. Cocci killed by heat can still provoke pus-formation.

Pathogenicity for Man.—Experiments, as well as the facts of comparative pathology, show that man is more susceptible than the ordinary laboratory animals to staphylococcus infection. Garré (1885)[1] inoculated himself by rubbing a pure culture upon the uninjured skin of the forearm, with the result that a series of carbuncles was produced, seventeen scars remaining to testify to the success of the experiment. His experiments have been repeated and confirmed by other observers (Bockhart,[2] Kaufmann[3]). The penetration of the cocci into the deeper layers of the intact skin, probably through the sweat-ducts or at the base of the hair-follicles, is a fact of considerable significance. The positive occurrence of such penetration seems well established, and the negative observations of some authors may well be referred to differences in the virulence of the strains employed or to other experimental discrepancies.

The demonstration that staphylococci have power under certain circumstances to penetrate the skin, taken together with their practically constant presence upon the skin itself, serves to explain the multiplicity of human affections with which these micro-organisms are found associated. A momentary weakness on the part of the tissues in almost any locality may lead to a rapid local invasion, followed by the production of a simple boil or by a more or less

[1] Garré: Fortschr. d. Med., 1885, 3, p. 165.

[2] Bockhart: Baumgarten, "Lehrbuch der path. Mykologie," Braunschweig, 1890.

[3] Kaufmann: Baumgarten's Jahresb., 1900, 16, p. 110.

extensive carbuncular condition. Septicemia and pyemia sometimes result through the introduction of staphylococci into the lymphatics or the blood-stream from a local abscess. The localization of the secondary foci varies in different cases. In the series of cases studied by Otten[1] 25 per cent. developed endocarditis. Sometimes general sepsis ("staphylomycosis") results from the most trivial local focus, such as a small boil or slight skin wound.

Staphylococci are not only found frequently in all parts of the body in secondary and mixed infections, but they are also primarily responsible for a variety of specific pathologic conditions and for injury to particular organs. Many lesions and diseases of the skin have been attributed to staphylococci; in the case of some of these it has been claimed that special varieties or races are concerned, but the characters said to distinguish these from the ordinary Staphylococcus aureus or albus are not, as a rule, of differential value.

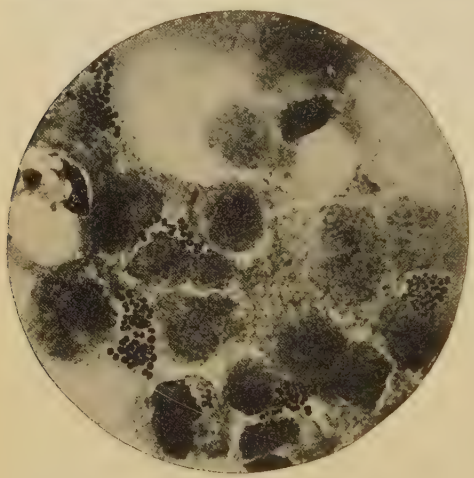

Fig. 34.—Staphylococcus aureus in pus from human abscess. Fuchsin stain; × 1000 (Fränkel and Pfeiffer).

A considerable majority of all attacks of acute osteomyelitis and periostitis are due to staphylococci, which appear to have a special predilection for the tissues of the osseous system.

Suppurative inflammation,[2] in whatever part of the body it may occur, is usually attended with the presence of staphylococci either in pure or mixed cultures (Fig. 34). Sometimes when found in a mixed infection they are doubtless the original exciting cause; in other cases they may have arrived at the seat of trouble only

[1] Otten: Deutsch. Arch. f. klin. Med., 1907, 90, p. 461.

[2] Suppurative inflammation is characterized particularly by the greatly increased immigration of the polymorphonuclear leukocytes into the affected part, by the lack of any coagulating power in the fluid portion of the pus (absence of fibrinogen), and by the necrosis and subsequent more or less complete digestion of the neighboring tissue elements.

after a primary invasion by some other microbe. In a given instance it may be impossible to determine the precise sequence of events.

Staphylococcus infection of the lung sometimes occurs, and the resulting bronchopneumonia is highly fatal. Out of about 800 cases of pneumonia treated at the hospital of the Rockefeller Institute in New York City from 1913–18, 13 were cases of staphylococcus infection, and 10 of these died. Under certain conditions, as during the influenza epidemic at Camp Jackson, Staphylococcus aureus has apparently played an important part in the pneumonia complicating primary infections. Chickering and Park[1] found that in 49 per cent. of 312 postmortem lung cultures at Camp Jackson this organism was present either alone (92 cases) or in association with other bacteria. Its presence in the lungs is usually interpreted as a secondary invasion in the train of some primary exciting agent.

Pathogenicity for Other Animals.—The rabbit has proved one of the more favorable animals for experimentation, intravenous injection of broth cultures being the most successful mode of infection. A moderately virulent strain kills an average-sized rabbit in four to eight days after injection of $\frac{1}{10}$ c.c. of a one-day broth culture (Neisser and Lipstein[2]). On autopsy minute abscesses are found in various internal organs, most commonly in the kidney (especially in the cortex of this organ) and in the walls of the heart. Under ordinary conditions of experiment with healthy adult rabbits the bone-marrow and periosteum are rarely seriously affected. In young animals, however, several experimenters claim to have evoked typical osteomyelitis by intravenous injection of staphylococcus cultures. It is perhaps questionable whether in these cases the processes in the affected tissues are strictly comparable with natural osteomyelitis in man. The injection of cultures into a rabbit suffering from a fractured bone or an injured periosteum produces a more characteristic train of events, and one that resembles closely the course of human osteomyelitis. Rabbits are relatively insusceptible to peritoneal inoculation with staphylococci. Artificial inoculation of the eye, on the other hand, succeeds readily, although natural eye infection is never observed. Feeding experiments with staphylococci give negative results. White mice are sometimes used for inoculation experiments, but are less uniformly

[1] H. T. Chickering and J. H. Park: Jour. Amer. Med. Assoc., 1919, 72, p. 617.

[2] Neisser and Lipstein: Kolle and Wassermann, Handbuch, 3, p. 126.

responsive than rabbits; guinea-pigs are relatively resistant, rats and pigeons highly so.

Cases of spontaneous staphylococcus infection among domestic animals, while not so common as in man, are not unknown. In horses and cattle Staphylococcus aureus has been found associated with pathologic processes and conditions similar to those that it produces in human beings. Some observers believe that they have discovered special species of staphylococci in certain animal affections, *e. g.*, "Staphylococcus (pyogenes) bovis" (in cattle) and "Staphylococcus hæmorrhagicus" (in sheep). Typical strains of Staphylococcus aureus and albus have been isolated from spontaneous abscesses in birds. A definite disease of the house fly has been traced to a specific staphylococcus (St. muscae).[1]

Varieties.—Attempts have been made to distinguish varieties of staphylococci on the basis of certain cultural reactions. The differences in pigment formation on which emphasis was laid for a time by some writers are probably of little significance, and the group of staphylococci must be regarded as a relatively homogeneous one.

The strains that produce white pigment (St. albus) are, as a rule, less active in gelatin liquefaction and fermentative power, and hence have been sometimes regarded as weakened relatives of the biochemically more vigorous orange pigmented types (St. aureus). Glycerol, however, seems to be fermented more rapidly by the St. albus strains. Lactose and mannitol are fermented by the majority of orange strains and by a small proportion (especially mannitol) of the white. In general, the types most commonly isolated from air, dust, and other sources outside of the human body are white staphylococci, while those found associated with pathologic conditions are orange strains.

The use of agglutination and other serologic reactions for differentiating varieties of staphylococci has not as yet led to any very satisfactory conclusions. The agglutinative power of a serum is usually so much more powerful for the strain used for immunizing than for other strains, whether these possess the same cultural characters or not, that immunologic relationships are not easy to trace. Some experimenters believe that "pathogenic" and "saprophytic" races can be distinguished on the basis of agglutination reactions, but such a classification has never been fully worked out.

[1] Glaser: Am. Jour. Hyg., 1924, 4, p. 411.

An interesting race of the albus variety (Staphylococcus epidermidis albus, Welch) is found in the deeper layers of the human skin, where it is not reached by ordinary methods of disinfection. It is of relatively slight virulence, and is the cause of the "stitch abscesses" following certain surgical proceedings. The ordinary Staphylococcus albus type is found not uncommonly in abscesses in man, sometimes in pure culture, more often in conjunction with the aureus variety. Staphylococcus (pyogenes) citreus is a rarer form of doubtful pathogenicity, producing a lemon-yellow pigment, but in other respects standing close to Staphylococcus aureus. Two "pigment varieties" of staphylococci still more infrequently met with in human pathology are Staphylococcus cereus albus and Staphylococcus cereus flavus; these forms do not liquefy gelatin; they produce a wax-like growth on the surface of the medium.

Immunity.—Rabbits may be made actively immune against intravenous injection with staphylococci by inoculating them first with killed, then with living but attenuated, cultures. During the course of this treatment, however, the animals sometimes succumb, and it is evident that the incorporation of staphylococcus cells into the body is attended with some danger to the subject of experiment. If filtrates containing staphylococcus hemolysin and leukocidin be used, antibodies for these toxins are formed; but immunity certainly does not depend upon the presence of these antibodies, since no connection has been shown to exist between increased resistance and the presence of such antibodies in the blood. Neither does prolonged immunization with the staphylococcus cells and their products cause any appreciable increase in the amount of bacteriolytic substance.

The production of active immunity to staphylococcus is, however, accompanied by a striking development of phagocytic action. Phagocytosis unquestionably plays the chief rôle in immunization to this organism. Staphylococci injected into an immunized animal are much more rapidly taken up by the phagocytes than is the case in a normal animal. Such increased phagocytosis, again, has been shown by Wright and others to be connected with the formation of bacteriotropic substances or opsonins in the blood of immunized animals. Methods of treatment based upon the increase in the amount of opsonin, and carried out by inoculation with a "vaccine" consisting of three-weeks-old broth cultures, killed

by heating at 60° C. for an hour, have been very successfully applied by Wright and others to the treatment of obstinate cases of acne and furunculosis in man. Hartwell and Lee,[1] for example, conclude that treatment with vaccine is the most effectual remedy for boils and carbuncles and for cases of chronic furunculosis. These authors state that the vaccine treatment can be successfully carried on without estimation of the opsonic index, but others maintain that it should not be used without accurate opsonic control. There is good evidence that the autogenous staphylococcus vaccine (the strain cultivated from the patient) is more efficacious than the ordinary stock vaccine.

Injection of the serum from an immunized animal will protect untreated animals against infection; in this case, also, the acquired immunity (passive) seems to be associated with an increase in the amount of opsonin.

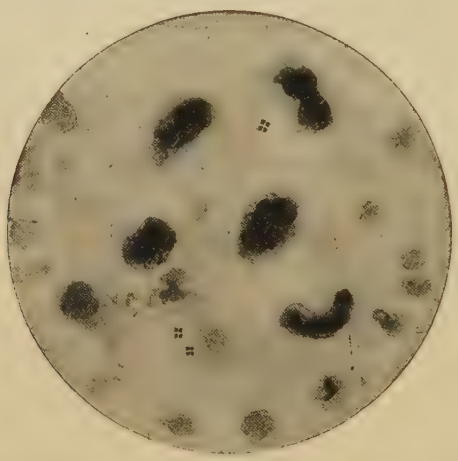

Fig. 35.—Staphylococcus tetragenus from peritoneal pus (Bythell).

Staphylococcus (Micrococcus) Tetragenus.—This organism was discovered by Gaffky[2] in the pulmonary cavities in phthisis. It has also been found in pure culture in abscesses in animals and man.[3] It often occurs in the healthy mouth. Morphologically St. tetragenus is distinguished by its occurrence in tetrads or groups consisting of four small oval cocci; it is gram-positive (Fig. 35). In cultures the sheet-like arrangement is not always seen, but in the

[1] Hartwell and Lee: Bost. Med. and Surg. Jour., 1907, 157, p. 523.
[2] Gaffky: Mitt. a. d. k. Gesund., Ber., 1881, 1, p. 1.
[3] Müller: Wien. klin. Wchnschr., 1904, 17, p. 815.

animal organism the flat tablets occur uniformly, and a rather heavy capsule surrounds the tetrad. On agar a confluent, rough, elevated white growth is produced. On potato a thick, white, slimy growth occurs. Gelatin is not liquefied; milk is coagulated.

Growth is slow and occurs both at 20° and at 37° C., though better at the higher temperature.

White mice inoculated with St. tetragenus succumb to a rapidly progressing septicemia. Guinea-pigs and rabbits usually show only a local affection. House-mice and rats are rather immune. Fornaca[1] has reported a case of septicemia in man in which St. tetragenus was present in pure culture in the blood. It is not uncommonly found in suppurations of the mouth and neck. It is also found in the empyema following pneumonia and in the pus of war wounds.

This organism is probably of a low-grade virulence, and unable, as a rule, to invade the human tissues except when the resistance is lowered by some depressing influence, especially of the kind caused by the invasion of some other micro-organism.

[1] Fornaca: Rif. Med., 1903, 19, p. 309.

CHAPTER X

THE STREPTOCOCCI

Genus: Streptococcus. Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). Generally stain by Gram. Capsules rarely present, no zoögleal masses. On agar streak, effused translucent growth, often with isolated colonies. In stab-culture, little surface growth. Many sugars fermented, with formation of large amount of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates.

Type species is Streptococcus pyogenes, Rosenbach.

Streptococci, as well as staphylococci, were seen long ago by several observers in the pus formed during suppurative inflammation, but their constant presence and pathologic significance were first strongly emphasized by the work of Ogston (1881),[1] Fehleisen (1883),[2] and Rosenbach (1884).[3] Owing to the close resemblance of streptococci from various sources and despite the great variety of pathologic conditions with which they were found associated, it was not at first known whether the various conditions were due to one or to several species. The trend of recent research has been to establish the existence of distinct types. The streptococci of scarlet fever, of erysipelas and of septic sore throat are now recognized as specific microbes even though it is still difficult to effect a differential diagnosis on purely morphological or cultural grounds.

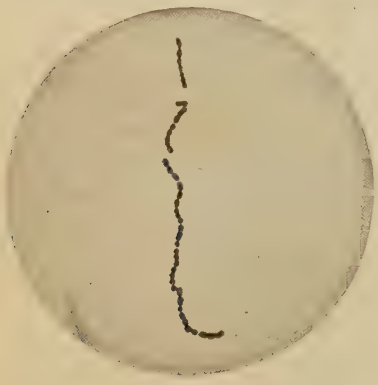

Fig. 36.—Streptococcus pyogenes. Pure culture in bouillon. Fuchsin stain; × 1000 (Kolle and Wassermann).

Morphologic and Cultural Characters.—The cells of streptococcus, like those of staphylococcus, are spherical, but differ from those

[1] Ogston: Brit. Med. Jour., 1881, 1, p. 369.

[2] Fehleisen: "Aetiol. d. Erysipels," Berlin, 1883.

[3] Rosenbach: "Mikroorganismen bei d. Wundinfektionskrankheiten," Wiesbaden, 1884.

of the latter organism in being usually united in longer or shorter chains (Figs. 36 and 37). Under certain conditions, however, they are aggregated in irregular heaps or masses. The diameter of the individual cocci is about 1 μ, although some variation is noted according to the character of the culture-medium, and smaller cells are not rare. The typical streptococcus always divides in one plane, so that if the cells remain united, a typical chain results. Transition forms between this and the staphylococcus have, however, been observed. Streptococci are not motile under the ordinary conditions of observation, do not possess flagella, and do not form spores. The streptococci isolated from pathologic processes in man retain the stain by Gram's method, but some streptococci found in suppurative conditions in domestic animals are said to be gram-negative.

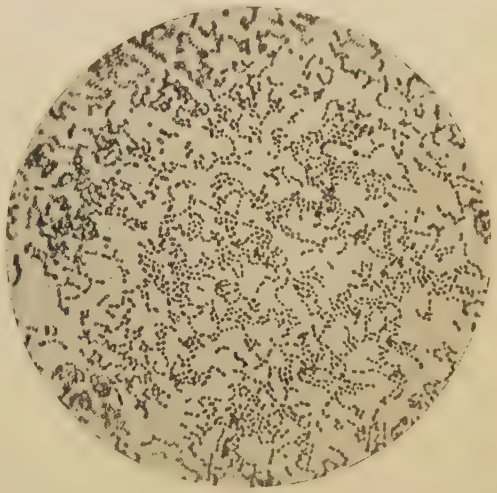

Fig. 37.—Streptococcus, pure culture (Moser and v. Pirquet).

In the opinion of some investigators an important distinction should be drawn between long-chain (Streptococcus longus) and short-chain (Streptococcus brevis) streptococci. The former are thought to be more virulent, and, as a matter of fact, the streptococci freshly isolated from disease processes in man usually grow out into long chains (of more than eight cells), while saprophytic streptococci, such as are commonly met with in the normal mouth and throat, develop short chains. Other morphologic and biologic qualities are believed to correspond. It has been found possible, however, to transform the long-chain into the short-chain variety by alteration of the culture-medium, and, furthermore, short-chain streptococci which are virulent are sometimes isolated from pathologic cases. As an absolute distinction, therefore, any fundamental separation into Streptococcus longus and Streptococcus brevis breaks down, but

as calling attention to a generally valid correlation character, the names have some value.

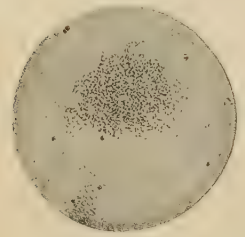

Fig. 38.—Streptococcus pyogenes. Colony on glycerol-agar, one day; × 100 (Heim).

Upon ordinary nutrient agar and gelatin streptococci, as a rule, yield but a scanty growth of fine, transparent, separate colonies (Figs. 38 and 39). Development is much facilitated, however, by the addition of dextrose (0.5 to 1.0 per cent.) to the medium. The common pathogenic strains do not liquefy gelatin, but some of the saprophytic streptococci, isolated from the alimentary tract and from polluted water, possess liquefying power. The growth in broth varies in different specimens; it is sometimes fine and powdery, sometimes coarse and flocculent. The character of the sediment that collects at the bottom of a broth culture has been supposed to give some indication of the essential nature of the organism producing it. Differentiation on this basis has not, however, been generally accepted. It is true that the long-chain forms usually give rise to distinct granules (Streptococcus conglomeratus), but it has not yet been shown that an invariable diagnostic value can be attached to the characters of broth cultures. Blood-agar plates are useful for the differentiation of streptococci. (See Classification, p. 209.) Streptococci that produce a green coloration on blood-agar plates are very similar to pneumococci, but, as a rule, do not ferment inulin, whereas most pneumococci attack this carbohydrate. A further distinction of great importance is that streptococci are insoluble, pneumococci soluble, in bile and solutions of bile salts. Blood-serum (horse, man) diluted with broth, or undiluted (rabbit), is a favorable medium for the maintenance of vitality and virulence. Growth occurs in milk, and is usually followed by curdling due to fermentation of the lactose, but some strains, particularly the long-chain varieties,

Fig. 39.—Streptococcus pyogenes. Agar culture, two days, showing colonies (Fränkel and Pfeiffer).

generate too little acid to effect any visible change. Upon potato a more or less luxuriant growth is produced by most saprophytic forms, but the pathogenic varieties develop sparingly, and many refuse to grow at all. The preservation of streptococcus cultures, often a matter of some difficulty, is best effected by maintaining either serum-broth mixtures or gelatin stab-cultures at a low temperature (8° to 10° C.).

Classification of Streptococci.—The early attempts to differentiate streptococci by means of cultural reactions were accompanied with much confusion. This was largely due to a lack of uniformity in the methods employed by different observers and to the use of an insufficient number of differential tests. One of the most important aids in the study of streptococci has been the blood-agar plate method introduced by Schottmüller in 1903.[1] This method has been subjected to elaborate study by J. H. Brown[2] and others.[3] The blood-agar plate has led to a division of streptococci into green-producing ("Streptococcus viridans") and hemolytic ("Streptococcus hemolyticus") strains, a distinction which is regarded as of primary significance.

The fermentation of certain carbohydrates has also been considered to give information of differential value and has been widely used since the method was first introduced by Gordon[4] in 1903. Unfortunately, some of the earlier work on carbohydrate fermentation was carried out with unsuitable media and without reference to the primary distinction between green-producing and hemolytic strains.

(*a*) *Hemolytic Streptococci* (Type Beta—Smith and Brown).—On suitably prepared blood-agar plates the hemolytic strains are characterized by a sharply defined clear zone around the colonies. It seems doubtful whether this group is sufficiently homogeneous to justify the use of the specific name Streptococcus hemolyticus for all its members. Until definite data are available a more general designation seems preferable. The great majority of the strepto-

[1] H. Schottmüller: Münch. med. Wchnschr., 1903, 50, p. 849.

[2] J. H. Brown: The Use of Blood Agar for the Study of Streptococci, Monograph No. 9, The Rockefeller Institute for Medical Research, 1919.

[3] H. W. Lyall: Jour. Med. Res., 1914, 30, p. 487; W. L. Holman: Jour. Med. Res., 1916, 34, p. 377; F. G. Blake: Jour. Med. Res., 1917, 36, p. 99.

[4] M. H. Gordon: Suppl. Annual Report Local Government Board, 1902–03, p. 421.

cocci found in pathologic processes are of the hemolytic type. Nearly all hemolytic strains ferment lactose and salicin; very few ferment mannit.

There is conclusive evidence that biologically different strains of hemolytic streptococci exist. According to Avery and Cullen bovine hemolytic streptococci cease to grow at a higher hydrogen ion concentration (4.5–4.3) than do human strains (5.2–5). Dochez, Avery and Lancefield[1] in a study of 125 human hemolytic strains showed that four and perhaps six biological types could be distinguished by means of the reactions of agglutination and protection. Ruth Tunnicliff[2] found that the serum of a sheep immunized with a hemolytic streptococcus from the throat of a patient in the acute stage of scarlet fever contained opsonins and agglutinins for hemolytic streptococci obtained from other scarlet fever cases, but not for hemolytic streptococci from other sources, such as erysipelas, measles, influenza, and diphtheria. Similar results were obtained by Bliss.[3] It has also been shown by Ruth Tunnicliff[4] that the serum of a sheep immunized with hemolytic streptococci from scarlet fever protects mice against strains of hemolytic streptococci isolated from scarlet fever patients, but not against strains from other sources. The results of tests by the same investigator indicate that the hemolytic streptococci from erysipelas likewise form a distinct immunologic group.

(*b*) *Green-producing Streptococci* (Type Alpha—Smith and Brown).—These organisms produce a greenish zone about the colony on blood-agar plates, the green coloration being due probably to a reduction process which converts the oxyhemoglobin into methemoglobin.[5] The name Streptococcus viridans has been proposed for the green-producing streptococci, but there is perhaps even more reason than in the case of the hemolytic streptococci for regarding the green streptococci as composed of several groups. The designation enterococcus is used by most French writers for the streptococci of the human intestine (Str. fecalis). Definite fermentation

[1] A. R. Dochez, O. T. Avery and R. C. Lancefield: Jour. Exp. Med., 1919, 30, p. 179.

[2] Ruth Tunnicliff: Jour. Amer. Med. Assoc., 1920, 74, p. 1386.

[3] W. P. Bliss: Bull. Johns Hopkins Hosp., 1920, 31, p. 173.

[4] Ruth Tunnicliff: Jour. Amer. Med. Assoc., 1920, 75, p. 1339.

[5] The so-called indifferent strains were found by Clawson (Jour. Infect. Dis., 1920, 26, p. 93) to be, in most if not all cases, methemoglobin producers when grown on suitable media.

reactions, as a rule, characterize strains from certain sources, and specific names have been given to such strains by a number of investigators. The classification by Blake[1] is an example of such a division according to fermentive properties.

GREEN-PRODUCING STREPTOCOCCI

Name.	Lactose.	Mannite.	Habitat.
Str. buccalis	+	−	The most common type in the human mouth and throat.
Str. fecalis	+	+	The most common type in human feces.
Str. equinus	−	−	The most common type in horse feces.

The green-producing streptococci are for the most part of much lower pathogenic power than the hemolytic strains, although at times highly virulent green strains have been isolated. The 1918 influenza epidemic was marked by the appearance of such strains in certain localities.[2] It may be fairly concluded that the group of green streptococci contains a number of organisms of potential pathogenic power as well as numerous saprophytic types, and that it deserves further intensive study.

Virulence (Toxin Production), Hemolysin.—The virulence of different strains of streptococci varies widely, but the factors upon which such virulence depends have not been discovered. Virulence for one species may be greatly exalted by animal passage; at the same time the virulence of the same strain for another species may be diminished. There is evidence that streptococci which are isolated directly from septic processes in man are more dangerous to man than similar organisms which have been living as saprophytes on the skin or mucous membrane. As a rule, growth within an animal body enhances the virulence of a microbe for that particular species, and this seems to be especially true in the case of the bacteria of blood-poisoning (streptococci and staphylococci).

The cell-substance of streptococci possesses only slight toxicity, and virulent strains do not differ from avirulent in this respect.

Old filtrates are more or less toxic, but no powerful specific toxin has been demonstrated. It is possible that certain poisons are

[1] Blake: Jour. Med. Res., 1917, 36, p. 99.

[2] Ruth Tunnicliff: Jour. Amer. Med. Assoc., 1918, 71, p. 1753; E. O. Jordan: Jour. Infect. Dis., 1919, 25, p. 28; E. C. Rosenow: Jour. Amer. Med. Assoc., 1919, 72, p. 1608.

formed by streptococci in the living body which are not produced in cultures.

A specific hemolysin, streptolysin, has been demonstrated by Besredka,[1] Ruediger, and others.[2] According to Ruediger,[3] streptolysin is a true toxin, containing a haptophore and a toxophore group, and giving rise, on injection, to a specific antibody. There is no good evidence that this hemolytic substance bears any constant relation to the virulence of the micro-organism producing it, or that it plays any part in producing the pathologic conditions caused by streptococcus infection.

A toxic substance that destroys leukocytes—leukocidin—is also produced by some strains of streptococci.[4] It is thought that there is a definite relation between the amount of leukocidin produced and the virulence of the strain.

Pathogenicity for Man.—Few, if any, pathogenic organisms can lay claim to wider or more multifarious activities than the streptococci. The list of human diseases and affections with which streptococci are associated as the main and primary cause is already a long one, and is perhaps not yet complete. In addition to their conspicuous rôle as initiators of very diverse pathologic conditions, streptococci are present in "mixed infections" and "secondary infections" more often than any other microbes; that is to say, they have a tendency to follow in the wake of and act as accomplices to other pathologic organisms. It has been found from an examination of the heart's blood of cadavers that in about one-third of all fatal diseases streptococci invade the blood before death, and in these cases they perhaps aid more or less in facilitating a fatal termination.[5]

Many *suppurative inflammatory conditions* in different organs of the body are caused by streptococci (Fig. 40). Osseous tissues and tissues surrounding the bones are attacked less frequently by streptococci than by staphylococci, but the joints and serous membranes are often invaded.

Focal infection is often due to streptococci. This name is given to a systemic or local disease due to bacteria carried in the blood-

[1] Besredka: Ann. de l'Inst. Past., 1901, 15, p. 880.
[2] P. H. de Kruif and P. M. Ireland: Jour. Infect. Dis., 1920, 26, p. 285.
[3] Ruediger: Jour. Amer. Med. Assoc., 1903, 41, p. 962.
[4] Y. Nakayama: Jour. Infect. Dis., 1920, 27, p. 86.
[5] Simmonds: Virchow's Archiv, 1904, 175, p. 418.

or lymph-stream from a focus of infection.[1] The usual sites of primary foci of infection are about the head, as in the nasal passages, the tonsils, the middle ear, and the teeth. From these primary foci the streptococci or other organisms may find lodgment in a distant organ. The connection between an attack of tonsillitis and a coincident or subsequent attack of rheumatism has long been known. Appendicitis, iritis, and a variety of other diseases are probably sometimes caused by strains of streptococci carried in the blood-stream from foci of infection in the tonsils, teeth, or sinuses.[2] The eradication of the primary foci of infection, such as removal of the teeth or tonsils, has often been followed by rapid disappearance of long-standing joint infections and other pathologic conditions.

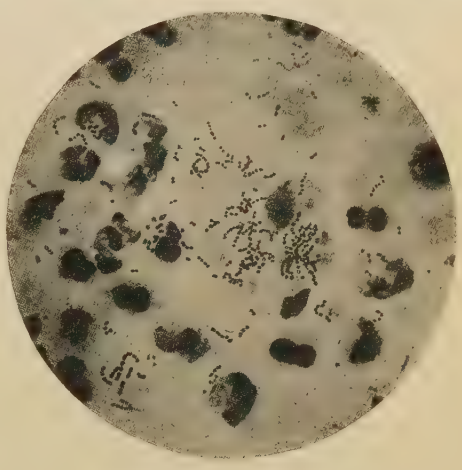

Fig. 40.—Streptococcus pyogenes in pus from a human abscess; gentian-violet (Fränkel and Pfeiffer).

Ulcerative endocarditis, an affection of the valves of the heart, which occasionally occurs simultaneously with inflammatory conditions in other parts of the body, is produced most frequently by streptococci, although it may also be caused by staphylococci, and less commonly by other micro-organisms. Experimentally the injection of broth cultures of streptococci (or staphylococci) into the circulation rarely produces ulcerative endocarditis unless the cardiac tissues have been damaged by mechanical or chemical agencies.

Süpfle[3] has found streptococci much more abundant than any other micro-organism in inflammation of the middle ear (otitis media).

The mucous membranes constitute a favorite abiding-place for streptococci: the tonsils almost always harbor them. Conse-

[1] Frank Billings: "Focal Infection," New York, 1916.

[2] E. C. Rosenow: Jour. Infect. Dis., 1915, 16, p. 240; Ibid., 1915, 17, p. 403.

[3] Süpfle: Centralbl. f. Bakt., I, Orig., 1906, 42, p. 304.

quently any lowering of the normal resistance of these tissues from either local or general causes gives the signal for a speedy invasion. In throat affections of all sorts streptococci are usually present, and in some cases, notably in diphtheria and in the anginas of scarlatina and measles, the part played by these organisms is highly important. Typical diphtheria is so generally accompanied by a multiplication of throat streptococci that at one time these microorganisms were regarded as the cause of diphtheria. No one now believes that streptococci cause diphtheria, but there is little doubt that, as accessories to the diphtheria bacillus, they do considerable harm. There is even some reason to believe that the virulence of the diphtheria bacillus itself is exalted in their presence.

The majority of non-diphtheritic anginas, in the opinion of many observers, are due directly to streptococcus infection. It cannot be said, however, that this position is entirely justified, since the facts are open to the interpretation that the streptococci found in simple anginas are merely secondary invaders which, as in diphtheria, have followed in the train of some primary infecting agent.

Hemolytic streptococci from the nose or throat of a surgeon or attendant may be the cause of wound infection, and complete masking is advocated as an important feature of operating room technic.[1]

The septicemic condition known as *puerperal sepsis* or childbed fever is usually to be attributed to streptococci. The once common hospital epidemics of puerperal septicemia, which fill so grim a page of medical history,[2] were probably due to the manipulative transfer of streptococci from infected cases; but it is also supposed that in some cases auto-infection occurs. Most observers agree that

[1] Cf. Meleney: Jour. Amer. Med. Assoc., 1927, 88, p. 1392.

[2] As long ago as 1843 Oliver Wendell Holmes published (New Eng. Quar. Jour. of Med. and Surg., April, 1843) an essay on the contagiousness of puerperal fever in which he marshals a surprising array of cases of this disease that were evidently carried to the mother by the attending physician or nurse. Some years later Semmelweis, in 1861 ("Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers," Pest, Wien u. Leipzig, 1861), showed clearly that puerperal mortality could be greatly reduced by attention to cleanliness, especially of the instruments and hands of the operator; but his teachings were for a long time neglected and even scorned. During the period before aseptic and antiseptic methods came into use in lying-in hospitals infection was conveyed from one case to another, and in some years nearly all the patients that entered a given hospital would die soon after being delivered of child.

streptococci are found in the vaginal secretions of pregnant women, especially when the secretions are alkaline. The prevailing opinion is that infection may take place with germs from any portion of the genital tract, including the external parts, and that special care should be given to the disinfection of the external genitalia before and during birth.

Certain cases of *enteritis* in infants and occasionally in adults have been attributed to streptococci (Escherich et al.). There is little doubt that streptococci, if not the primary cause of intestinal disturbance, are at least actively concerned in the pathologic processes. Several varieties of intestinal streptococci have been described, separable, it is said, from one another and from the ordinary Streptococcus pyogenes by more or less definite cultural characteristics; but the evidence showing a connection of any particular variety with the causation of intestinal disease is not so clear as could be desired. Intestinal infection of infants might conceivably occur through the medium of the milk-supply, since streptococcic inflammation of the mammæ (mastitis) of the cow is not uncommon. Streptococci have been often found in market milk, but it has not been shown that their presence, even in considerable numbers, is any indication that the milk is unwholesome. Most of the streptococci in milk are probably descended from saprophytic, not from pathogenic, ancestors. (See p. 630.)

As secondary invaders streptococci have a baneful influence in a number of maladies, notably in the last stages of pulmonary tuberculosis, in which they produce frequent complications, involve healthy tissues adjacent to the tuberculous area, and predispose to hemorrhages. Streptococci are often present mixed with pneumococci in pneumonia and in some cases act as the primary cause. In smallpox it is believed that many of the most serious symptoms and most frequent complications are the result of streptococcus infection.

Rheumatic fever or acute articular rheumatism has been attributed by different observers to various kinds of bacteria. An anaërobic bacillus found by Achalme[1] was at one time regarded by some writers as the cause of this affection, but Achalme's bacillus is probably identical with Cl. welchii (p. 403) and has no causal relation to rheumatism. Streptococci have been found in the

[1] Achalme: Arch. de méd. exper., 1898, 11, p. 370.

blood in a number of cases of rheumatic fever, and characteristic arthritic lesions have been produced in rabbits by inoculation with cultures derived from such cases. By a number of observers the streptococci (or diplococci) from acute rheumatism have been considered different from the ordinary Streptococcus pyogenes. Some investigators long ago reported that distinctive and constant cultural characters, such as the production of acid and precipitation of bile salts in MacConkey's bile-salt-lactose-broth, and abundant production of formic acid (Walker and Ryffel[1]), characterize the micrococcus found in rheumatism. Their early observations, however, were not promptly followed up. The discovery of specific streptococci in scarlet fever and in erysipelas stimulated the study of rheumatic fever, and Birkhaug[2] has described in detail a streptococcus with quite distinctive characters that he has isolated from patients with rheumatic fever. This organism neither hemolyzes nor produces a green coloration (methemoglobin) in blood agar; it ferments inulin and is insoluble in bile. Birkhaug, who isolated a number of streptococci of different types from rheumatic fever patients found that a much larger number of toxin producers occurred among the non-methemoglobin-forming streptococci than among the other strains. Persons with definite history of rheumatic fever reacted to intradermal injections of minute doses of the soluble toxic filtrate in far greater proportion than normal persons. Birkhaug, who injected himself with large doses of the soluble toxin filtrate of the suspected streptococcus developed "a typical clinical picture of acute polyarthritis of the rheumatic type."

In certain cases of *chronic arthritis* or arthritis deformans, hemolytic streptococci not readily distinguishable from the ordinary Streptococcus pyogenes have been isolated from the tonsil, often in pure culture.[3] These cultures invariably produce arthritis in animals. Extirpation of the diseased tonsil leads sometimes to marked improvement or to complete recovery. Injection of killed cultures of the streptococcus strain isolated from the patient (autogenous vaccine) was for a time widely used in the treatment of such cases, but observers with a wide experience in the employment of autogenous vaccines (Billings) are of the opinion that the therapeutic results do not warrant the use of these agencies.

[1] Walker and Ryffel: Brit. Med. Jour., 1903, 2, p. 659.

[2] Birkhaug: Jour. Infect. Dis., 1927, 40, p. 549.

[3] D. J. Davis: Jour. Amer. Med. Assoc., 1913, 61, p. 724.

Streptococcic septicemia may develop in connection with a great variety of affections, both those in which streptococci themselves are the initial exciting cause and those that are set in motion by other factors. The *coup de grace* in many prolonged constitutional maladies, such as diabetes, is often given through a general streptococcus invasion.

Among the American troops in the Great War hemolytic streptococci were frequently found as the primary or secondary invaders in cases of bronchopneumonia. The numerous fatal cases of pneumonia following measles in the army camps seem to have been almost always due to infection with hemolytic streptococci. A considerable proportion of these cases were accompanied by empyema in which hemolytic streptococci were likewise found. In certain localities the hemolytic streptococci, at first secondary invaders in the wake of the measles virus, seemed to acquire a higher degree of virulence and become able to initiate primary respiratory infection.

Besides causing typical erysipelas, streptococci sometimes give rise to other affections of the skin and lymph-vessels (*e. g.*, impetigo contagiosa[1]).

Erysipelas.—The pathogenicity of the streptococcus for man is well exemplified in erysipelas. This peculiar inflammation of the skin was shown by Fehleisen in 1883 to be due to streptococci. The cocci are not present in the central portion of the inflamed area, but are found on its periphery, and can be isolated most readily by excision of portions of the tissue, other methods rarely succeeding. In the skin they occur chiefly in the lymph-spaces, which are often packed with them. The organism may be recovered by skin puncture as far as 3 cm. beyond the advancing edge of the lesion where there is no gross evidence of inflammation. Erysipelas-like lesions in rabbits were produced by a number of early observers and have been extensively studied by later experimenters. Inoculation experiments made upon carcinomatous patients[2] have also dem-

[1] Kurth: Arb. a. d. k. Ges., 1893, 8, p. 294.

[2] Clinical observers have often noted that patients suffering from malignant tumor were distinctly benefited by an attack of erysipelas, that the growth was checked, and the tumor even diminished in size. Acting on this observation, Fehleisen ventured to inoculate streptococci into persons suffering from inoperable tumors, and the experiment was apparently rewarded with some degree of therapeutic success. Coley (Amer. Jour. Med. Sci., 1896, 112, p. 251) has modified the treatment by employing a mixture of killed

onstrated that pure cultures of erysipelas-streptococci can provoke the erysipelatous process.

It remained for a long time uncertain whether or not the hemolytic streptococci from erysipelas patients constitute a distinct biological type. The observations of Ruth Tunnicliff[1] were among the first to indicate a genuine typeless specificity, and later Birkhaug[2] found that over 90 per cent. of carefully isolated erysipelas strains fell into a single antigenic group, and differed from nonerysipelas strains. The latter observer has made important contributions to our knowledge. A skin reaction similar to that observed in the Schick and Dick tests is evoked by injection of culture filtrates into the skin of susceptible persons; the toxicity of the filtrates is neutralized both by convalescent erysipelas serum and by antiserum obtained through inoculation of animals (rabbit or donkey) with the specific streptococcus cultures; and highly encouraging results have been obtained in the treatment of erysipelas patients with specific immune serum.[3] Other observers[4] have come to the same conclusion as Birkhaug regarding specificity, and there can be little doubt that a more or less specialized type of hemolytic streptococcus is the cause of human erysipelas.

The early observations on immunity were somewhat at variance. Fehleisen could not always produce a second attack of erysipelas in persons who had been once successfully infected, but Koch and Petruschky[5] were able to cause as many as ten successive attacks in one individual in the same area of skin, a fresh inoculation being made each time immediately after an attack had subsided. The majority of experimenters, however, have noted some degree of immunity. Gay and Rhodes[6] state that recovery of rabbits from

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cultures of streptococci and B. prodigiosus, or the soluble products of these organisms. In all cases where an injection of dead or living streptococci has seemed to exercise a favorable influence, the sarcomatous tumors are most affected, the carcinomatous tumors being only slightly and temporarily inhibited. Many observers have been unable to note any favorable results following upon the use of "Coley's mixture."

[1] Ruth Tunnicliff: Jour. Amer. Med. Assoc., 1920, 75, p. 1339.

[2] Birkhaug: Bull. Johns Hopkins Hospital, 1925, 36, p. 248.

[3] Birkhaug: Jour. Amer. Med. Assoc., 1926, 86, p. 1411.

[4] *E. g.*, Stevens and Dochez: Jour. Exper. Med., 1926, 43, p. 379; ibid., 44, p. 439.

[5] Koch and Petruschky: Ztschr. f. Hyg., 1896, 23, p. 477.

[6] Gay and Rhodes: Jour. Infect. Dis., 1922, 31, p. 101.

erysipelas confers complete protection against intradermal reinoculation, irrespective of its locality, the protection usually lasting for at least three months. Amoss and Bliss[1] have found that in rabbit experimentation a local immunity develops on the inoculated sides of the animal while the non-injected side reacts normally. After many injections carried out over relatively long periods the non-injected side becomes resistant and at the same time there is evidence of general humoral immunity as shown by the presence of agglutinin and antitoxin in the blood. By repeated intracutaneous inoculation of rabbits with pure cultures, Rivers[2] succeeded in preparing a serum "capable of warding off the injurious action of the streptococcus on the skin." Normal serum had no such protective effect. It was also found[3] that a local passive immunity could be induced by infiltration with immune serum. The anti-erysipelas serum obtained by Birkhaug by the inoculation of larger animals with living streptococci according to the method used by Dochez in scarlet fever (p. 226) has been used in the treatment of erysipelas by Birkhaug and others and has given satisfactory results in a number of both moderate and severe cases. Singer and Kaplan[4], using artificially produced human immune serum, obtained by inoculation with sterile toxic filtrates, likewise noted evidence of protective power. Studies by Birkhaug[5] on the effect of repeated injection with soluble toxin have opened up possibilities of active immunization. "Twenty-four patients with definite histories of frequent recurrent attacks of erysipelas have been actively immunized by means of the toxic filtrates of Streptococcus erysipelatis, and persons among these who previously suffered habitual attacks of the disease from every sixth to twelfth week have been free from subsequent recurrent attacks over a period approaching two years."

Pathogenicity for the Lower Animals.—Among the lower animals streptococci are found in spontaneously produced abscesses and similar suppurative processes in about the same proportion as in man; they are less common than simple staphylococcus infections, but are said to comprise from one-third to one-fourth of all cases. Horses seem particularly subject to streptococcus infection. The

[1] Amoss and Bliss: Jour. Exper. Med., 1927, 45, p. 411.
[2] Rivers: Jour. Exper. Med., 1925, 41, p. 179.
[3] Rivers and Tillett: Jour. Exper. Med., 1925, 41, p. 185.
[4] Singer and Kaplan: Jour. Amer. Med. Assoc., 1926, 87, p. 2141.
[5] Birkhaug: Jour. Amer. Med. Assoc., 1927, 88, p. 885.

streptococci found in inflammation of the cow's udder are said to differ from the streptococci of human inflammations in being gram-negative, in their ability to liquefy gelatin, and in their higher pathogenicity for guinea-pigs.

Experimentally, rabbits and mice have proved most susceptible to inoculation. There is an entire absence of correlation in the pathogenicity of streptococci for different animals. A culture isolated from a severe septic infection in man may be utterly devoid of pathogenic power for the mouse, while a culture obtained from a small localized abscess may be highly mouse-virulent. Virulence for a given species of animal, such as the mouse, may be exalted by successive passages through individuals of that species, but the greatly heightened virulence thus obtained for one animal (mouse) may be accompanied by the simultaneous diminution of virulence for another (rabbit) (Knorr[1]). On the other hand, passage through rabbits increases the virulence not only for the rabbit, but also for the mouse and for the larger domestic animals. Cultures whose virulence has been artificially exalted for the rabbit and the mouse appear to have lost much of their pathogenic power for man. Rabbits and mice usually develop a generalized infection when inoculated with virulent strains. One-millionth of a cubic centimeter of a twenty-four-hour-old broth culture has been found to kill a rabbit, but such a high degree of virulence is rare except in artificially exalted cultures. As a rule, a culture is regarded as of fairly high virulence if 0.01 c.c. kills a rabbit within three or four days. Slightly virulent strains may produce localized abscesses; an erysipelatous process has been provoked in the ear of the rabbit by the use of a culture of the proper degree of virulence.

Streptococcus Sore Throat (Septic Sore Throat).—In the first decade of the twentieth century epidemics of sore throat of a severe and unusual type appeared in a number of localities in England and elsewhere.[2] In Colchester, England, 600 persons were affected; in Christiania, Norway, about 550. Still more extensive outbreaks have occurred in this country: in Boston[3] (1911), 1400 cases; in Baltimore[4] (1912), 1000 cases; in Chicago[5] (1911–12),

[1] Knorr: Ztschr. f. Hyg., 1893, 13, p. 427.
[2] See Savage: "Milk and the Public Health," The Macmillan Co., 1912.
[3] Winslow: Jour. Infect. Dis., 1912, 10, p. 111.
[4] Hamburger: Bull. Johns Hopkins Hosp., 1913, 24, p. 1.
[5] Capps and Miller: Jour. Amer. Med. Assoc., 1912, 58, pp. 1111, 1848.

10,000 cases (estimated), and in Concord, N. H.[1] (1912), 1000 cases.

The symptoms and complications have been strikingly similar in all the epidemics studied. Intense local hyperemia, with or without a grayish exudate, and enlargement of the cervical lymph-nodes are among the more characteristic manifestations. The joints are affected in many cases and the heart and kidneys seriously damaged. Pneumonia ending in fatal septicemia is often observed. "The most dangerous and remarkable complication was peritonitis, which was responsible for a great number of deaths" (Capps).

The disease may be spread both by milk and by direct contact.[2] In several epidemics occurring in the United States the infection was traced definitely to particular milk supplies. Although some secondary cases developed by contact, over 70 per cent. of the victims in certain outbreaks were users of milk from a single dairy. Attempts to connect the outbreaks with a definite diseased condition of the cattle furnishing the milk have not been successful in all cases, but in the Cortland (N. Y.) epidemic[3] acute udder inflammation was found in cattle in the implicated herd. It is possible that milk may become infected after collection through the agency of human carriers, but the massive and continuous infection occurring in some of the outbreaks indicates that the udders of the cows were infected.

Streptococci of a peculiar type have been isolated from milk, from characteristic sore throats, and from the peritoneal exudate in fatal cases, and were first subjected to thorough study by D. J. Davis[4] and Rosenow,[5] of Chicago, and later by Brown, Frost and Shaw.[6] The name Streptococcus epidemicus, first used by Davis, is commonly applied to these organisms. The species is thus described by Brown, Frost and Shaw:

"A streptococcus of round or slightly flattened elements, producing hemolyzed zones of the beta type in blood agar, rapidly hemo-

[1] Mann: Jour. Infect. Dis., 1913, 12, p. 481.

[2] An extensive contact outbreak of septic sore throat in the State of New York was reported by Winslow and Hubbard (Monthly Bull., N. Y. State Dept. of Health, Sept., 1915).

[3] North, White, and Avery: Jour. Infect. Dis., 1914, 14, p. 124.

[4] Davis: Jour. Amer. Med. Assoc., 1912, 58, p. 1852.

[5] Rosenow: Jour. Amer. Med. Assoc., 1912, 58, p. 773.

[6] Brown, Frost and Shaw: Jour. Infect. Dis., 1926, 38, p. 381.

lyzing blood corpuscles in fluid media, markedly pathogenic for mice, fermenting glucose, lactose, saccharose and salicin but not mannite, producing a final hydrogen ion concentration not above P_H 4.8 in glucose broth, failing to hydrolyze sodium hippurate, and probably always encapsulated when observed under suitable conditions, in the last respect differing from most strains of hemolytic streptococci isolated from sporadic human infections." The streptococci ordinarily present in certified milk are of harmless bovine type. In several instances, however, Streptococcus epidemicus has been found in persistent udder infection.[1] The animals so infected should be immediately removed from milk-producing herds.

It seems probable that the streptococci of this infection are primarily of a human rather than a bovine type, and that the udder of the cow is infected directly through a human source, as, for instance, from the hands of a milker. Davis has made the important observation that the udder of a healthy cow may be infected with streptococci of human origin which produce a mastitis unaccompanied by "caking" of the bag. Such a form of mastitis might well escape notice in the ordinary examination of a suspected herd.

"When we come to a consideration of prophylaxis all other measures and precautions will sink into insignificance when dompared with thorough pasteurization" (Capps).

Scarlet Fever.—The frequent presence of streptococci of various kinds in normal and diseased throats and the difficulty—which still exists—of effecting a differentiation by the usual culture methods delayed for a long time the general acceptance of a streptococcus as the cause of scarlet fever. The importance of streptococci as secondary invaders, and other factors such as the low and transient immunity conferred by many streptococcus infections as contrasted with the relatively lasting immunity left by an attack of scarlet fever were additional reasons why many bacteriologists hesitated to believe that this disease was due to a specific streptococcus. Some early observers (Moser, Ruediger) noted significant agglutination reaction (Fig. 41), and in May 1920 Ruth Tunnicliff[2] and Bliss[3] published independently observations showing that a hemo-

[1] Frost, Gumm and Thomas: Jour. Infect. Dis., 1927, 40, p. 698.
[2] Tunnicliff, Ruth: Jour. Amer. Med. Assoc., 1920, 74, p. 1386
[3] Bliss: Bull. Johns Hopkins Hospital, 1920, 31, p. 173.

lytic streptococcus found in large numbers in scarlet fever patients could be differentiated clearly and sharply by immunological reactions (agglutination, opsonification, protection) from the hemolytic streptococci obtained from other diseased conditions (erysipelas, measles, mastoiditis) and from normal throats. While there are difficulties in carrying out these tests[1] there is on the whole general agreement that the scarlet fever streptococci, although not serologically identical, constitute a distinct biological group.[2]

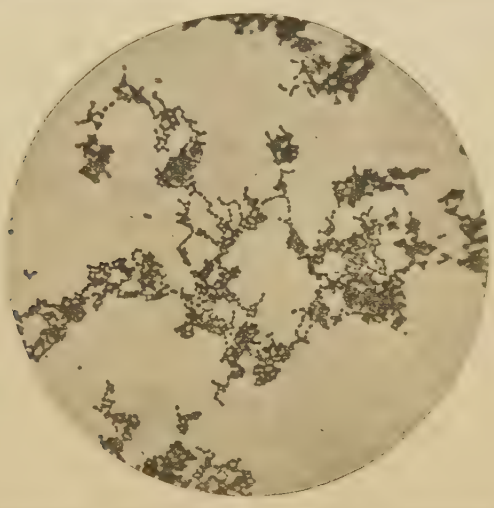


Fig. 41.—Streptococcus agglutinated by immune horse-serum (Moser and Pirquet).

The early attempts to infect the lower animals and man with streptococci isolated from scarlet fever failed to give unambiguous results. The reason may have been partly because not all the streptococci isolated from scarlet fever throats are necessarily of the specific type, and partly because Streptococcus scarlatinæ loses its specific qualities rather quickly on cultivation. Finally in 1923 G. F. and Gladys H. Dick succeeded in reproducing typical scarlet fever in human volunteers;[3] and Dochez and Sherman[4] evoked

[1] Cf. Stevens and Dochez: Jour. Exper. Med., 1924, 40, p. 253; Tunnicliff, Ruth: Jour. Amer. Med. Assoc., 1926, 87, p. 625.

[2] Cf. Griffith: Jour. Hyg., 1927, 26, p. 363; J. Smith: ibid., pp. 420, 434.

[3] G. F. and Gladys H. Dick: Jour. Amer. Med. Assoc., 1923, 81, p. 1166; Jour. Amer. Med. Assoc., 1924, 82, p. 301.

[4] Dochez and Sherman: Jour. Amer. Med. Assoc., 1924, 82, p. 542.

significant symptoms in guinea-pigs and shotes. These striking positive results with pure cultures were soon followed by the signal discovery by the Dicks[1] that the filtrates of the cultures that had produced experimental scarlet fever, when injected intracutaneously in the proper dilution, gave a distinct local reaction in the skin of a large proportion (41.6 per cent.) of persons who had no history of scarlet fever, while all of the convalescent scarlet fever patients tested showed negative or only slightly positive reactions. It was found further that the action of the filtrate on the skin was inhibited by small quantities of convalescent scarlet fever serum mixed with the filtrate before injection. These observations on the essential specificity of the skin test (Dick test) have been confirmed and extended by numerous observers in various parts of the world.[2] Pseudo-reactions and reactions of various types sometimes occur but apparently as a rule can be interpreted correctly by experienced observers. In the opinion of some, a negative Dick test cannot be considered a perfectly reliable index of immunity since in certain outbreaks a considerable proportion of patients may give a negative test at the onset of the disease.[3]

It seems, therefore, to be true that a toxin-producing hemolytic streptococcus, Streptococcus scarlatinæ, is the cause of scarlet fever and this view is widely though not universally accepted at the present time.

Recent attempts to discover some cultural or biochemical property other than immunological that separates Streptococcus scarlatinæ from other hemolytic streptococci have not been successful. The Dicks produced experimental scarlet fever both with mannite-fermenting and non-mannite-fermenting strains. The same observers[4] suggest that the neutralization of the toxin with specific antitoxin affords a satisfactory means of recognizing the scarlet fever streptococcus. Ruth Tunnicliff has found immune rabbit serum is equally specific and that direct opsonin tests permit ready differentiation.[5]

[1] G. F. and Gladys H. Dick: Jour. Amer. Med. Assoc., 1924, 82, p. 265.

[2] The analogy of the Dick test in scarlet fever with the longer known Schick test in diphtheria (p. 302) is very close; the underlying principle of the two reactions is doubtless fundamentally the same.

[3] Cf. Lees: Jour. Amer. Med. Assoc., 1927, 88, p. 1133.

[4] G. F. and Gladys H. Dick: Jour. Amer. Med. Assoc., 1925, 84, p. 802.

[5] Tunnicliff, Ruth: Jour. Amer. Med. Assoc., 1926, 87, p. 625.

As indicated by the Dick skin test there is reason to believe that Streptococcus scarlatinæ produces at least part of its pathogenic effect by means of a soluble toxin. The Schultz-Charlton blanching phenomenon had earlier pointed to the same conclusion although its significance was for a time not recognized.[1] When a scarlet fever patient with a bright rash is injected with 1 c.c. of serum from a patient convalescent from scarlet fever, the rash after about six hours begins to fade away, and soon disappears completely. Serum obtained from a patient in the active stage of scarlet fever lacks this blanching power. On the other hand serum from apparently normal persons often possesses the power to extinguish the rash, although the proportion of the general population having serum with this power is 60 per cent. as against 80–100 per cent. of known scarlet fever convalescents. The serum of young children not known to have had scarlet fever manifests a positive reaction in a much smaller proportion than adult serum. The most obvious interpretation of the Schultz-Charlton blanching phenomenon is that an antitoxic substance is present in the serum of persons who have had scarlet fever—whether the attack be recognized or not—or who have inherited maternal antibodies, and that this antitoxin neutralizes the circulating scarlet fever toxin, so causing the extinction of the rash.

Definite information on the presence and persistence of true scarlet fever streptococci in nose and throat and in the environment of patients is still very meager. J. E. Gordon[2] found that hemolytic streptococci—not necessarily all of them of the Streptococcus scarlatinæ type—remain on the mucous surfaces of the upper respiratory tract longer in severe than in mild cases. More than one-half of a group of scarlet fever patients studied in the Chicago Contagious Disease Hospital gave positive cultures at the time of release (four weeks); those that had received specific serum, however, harbored hemolytic streptococci in smaller proportion than the untreated. Patients with complications became carriers of hemolytic streptococci in larger numbers and for a longer time than those without complications. It is evident that an arbitrary period of isolation is no more likely to be effective in preventing the spread

[1] Schultz and Charlton: Ztschr. f. Kinderheilh., 1918, Orig. 17, p. 328.
[2] J. E. Gordon: Jour. Preventive Med., 1927, 1, p. 289.

of scarlet fever than it was of diphtheria (p. 278). Holst[1] considers that there is no evidence that the isolation of scarlet fever in Norway has materially diminished the frequency of the disease. It is to be hoped that a satisfactory bacterial procedure may soon be found so that a period of isolation based on the demonstrated presence or absence of the specific scarlet fever streptococcus may be substituted for a formal time requirement.

In their series of human inoculation experiments Dick and Dick reported sore throats without a rash in some volunteers and typical scarlet fever in others following inoculation with the same streptococci, and observations by Stevens and Dochez[2] have established the specific identity, long suspected by clinicians, of clinical scarlet fever and certain throat infections without exanthem; the same strain of Streptococcus scarlatinæ may give rise now to one, now to the other condition.

Curative Serum.—As early as 1902 Moser[3] immunized horses to streptococci isolated from scarlet fever patients and reported strikingly successful therapeutic results with the immune serum from these animals. Subsequent experimenters for the most part failed to obtain similar results, and the use of a specific serum for scarlet fever consequently did not come into general use until much later. Early in 1924 Dochez[4] reported the production of an antistreptococcic horse serum which later was found to be toxin-neutralizing, and Blake, Trask and Lynch[5] administered Dochez's serum to scarlet fever patients and noted its definite therapeutic value. Blanching appeared in the same way as after the use of convalescent serum and Moser's serum. Intracutaneous or intramuscular injections proved effective in blanching and in treatment, while intravenous injection did not. A specific scarlet fever antitoxin was prepared by the Dicks,[6] who immunized horses with sterile toxic filtrates instead of with living streptococcus cultures as was done by Moser and Dochez. Treatment with this serum likewise has proved highly successful. Concentration of the Dick serum has been

[1] Holst, P. M.: Jour. Preventive Med., 1927, 1, p. 279.

[2] F. A. Stevens and A. R. Dochez: Jour. Amer. Med. Assoc., 1926, 86, p. 1110.

[3] Moser, P.: Wien. klin. Wchnschr., 1902, 15, p. 1053.

[4] A. R. Dochez: Proc. Soc. Exp. Biol. and Med., 1924, 4, p. 184.

[5] F. G. Blake, J. D. Trask and J. F. Lynch: Jour. Amer. Med. Assoc., 1924, 82, p. 712.

[6] G. F. and Gladys H. Dick: Jour. Amer. Med. Assoc., 1924, 82, p. 1244.

carried out and permits the use of relatively small volumes. Observers in various parts of the world have reported results that are on the whole favorable to the serum treatment of scarlet fever,[1] although the mildness of the prevalent type of the disease makes it difficult to secure any large body of statistics as cogent as those recorded in diphtheria (p. 294). Some clinicians would restrict the use of scarlet fever antiserum to cases of severe type.

It seems possible that the inability of many of the earlier experimenters to repeat Moser's therapeutic success was due to the lack of suitable criteria for true scarlet fever streptococci, and to the fact that they did not have the means of recognizing and standardizing the antitoxic content of the serum. Some of them may have worked with hemolytic streptococci of banal types which could not have been expected to manifest any specific antigenic qualities. It is perhaps significant that Moser himself used about 20 different strains of streptococci in the preparation of his immune serum.

Prophylactic Inoculation.—Preventive inoculation with killed streptococcus cultures appears to have been practiced by Russian bacteriologists as early as 1906.[2] Mild symptoms were produced similar to those observed in scarlet fever. A single injection did not suffice to produce immunity, two to three inoculations being necessary. It was believed that a considerable degree of protection was obtained by this procedure. Korschun and Spirina[3] using a formalin vaccine have lately reported good results.

The discovery of the scarlet fever toxin offered an opportunity for protective immunization similar to that successfully utilized in diphtheria, and was early taken advantage of by the Dicks.[4] A standard toxin is necessary for the safe and effective application of this method and a definite procedure of standardization has been worked out.[4] Small, properly graduated injections of toxin continued until a negative skin test is obtained seem to confer a considerable degree of immunity. At the Durand Hospital in Chicago 125 nurses and others exposed to scarlet fever were treated with

[1] J. E. Gordon: Jour. Amer. Med. Assoc., 1927, 88, p. 382. Cf. Lenthe: Deutsch. med. Wchschr., 1927, 53, p. 313.

[2] Gabritschewsky: Centralbl. f. Bakt. 1906, I, Orig., 71, p. 719; Nikitin: Jour. Amer. Med. Assoc., 1926, 87, p. 2143.

[3] Korschun and Spirina: Seuchenbekämpfung, 1927, 4, p. 40.

[4] G. F. and Gladys H. Dick: Jour. Amer. Med. Assoc., 1924, 82, p. 544.

standardized toxin (3 doses) and no scarlet fever developed among them, while in the control uninoculated group of 34 exposed persons 7 cases of scarlet fever occurred.[1] Observers in various parts of the world have reported favorable results in combating scarlet fever by the Dick Method.[2]

[1] G. F. and Gladys H. Dick: Jour. Amer. Med. Assoc., 1924, 83, p. 84.

[2] *E. g.*, Bokay, V.: Abstr., Bull. Hyg., 1927, 2, p. 367; Chodzko: Bull. Off. Internat. d'Hyg. Pub., 1926, 18, p. 1295.

CHAPTER XI

THE PNEUMOCOCCI

Genus: Diplococcus. Parasites. Grow poorly, or not at all, on artificial media. Cells usually in pairs of somewhat elongated cells, often capsulated, sometimes in chains. Gram positive. Fermentive powers high, most strains forming acid in glucose, lactose, sucrose, and inulin.

Type species is Diplococcus pneumoniæ, Weichselbaum.

The micro-organism most commonly met with in acute inflammations of the lungs in man is a small micrococcus upon which a great variety of names have been bestowed; those in most general use are *Diplococcus pneumoniæ*, *Micrococcus lanceolatus*, or, more briefly, the *pneumococcus*. It is also known, after its discoverer, as *Fränkel's pneumococcus*.

In considering the relations of this organism to the production of pneumonia, it must be borne in mind that the name pneumonia is not restricted to absolutely uniform changes in one set of tissues, but that writers group under this name a variety of affections symptomatically and histologically distinct. Among the most generally recognized of these are: lobar (acute croupous) pneumonia, bronchopneumonia or lobular pneumonia, and capillary bronchitis (bronchiolitis). If one of the anatomic types—for example, lobar pneumonia, the common form in adults—be considered from the etiologic standpoint, it is found that at the present time in temperate climates acute lobar pneumonia in the vast majority of cases is caused by a lanceolate micrococcus, occurring in pairs or chains—the pneumococcus of Fränkel. A bacterial study of lobar pneumonia at the Hospital of the Rockefeller Institute extending over several years gave the following findings:

MICRO-ORGANISMS ASSOCIATED WITH LOBAR PNEUMONIA

	NUMBER OF CASES.
Pneumococcus	754
Streptococcus pyogenes	7
Hemophilus influenzæ	6
Mixed infection	6
Staphylococcus aureus	3
Friedländer bacillus	3
Streptococcus mucosus	1

For the most part the pneumonias produced by bacteria other than the pneumococcus are of the lobular type. Pneumonia, then, is not a disease either of constant anatomic character or of uniform etiology. One and the same organism can incite affections histologically dissimilar, such as the lobar, lobular, and bronchiolitic forms; and, on the other hand, apparently identical lesions may be produced by the action of different microbes.

Morphology.—The pneumococcus is typically a small, slightly elongated coccus, one end of which is pointed or lance shaped. It commonly occurs in pairs (diplococci). Variations both in grouping and in the size and form of the individual cells are frequently observed.

Fig. 42.—Diplococcus pneumoniæ in pure culture one day old. Gram stain. Weichselbaum prep. (Kolle and Wassermann.)

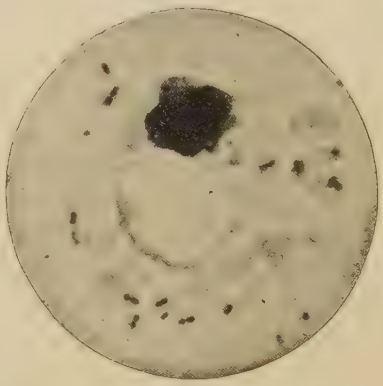


Fig. 43.—Diplococcus pneumoniæ in exudate from human lung; anilin-water-fuchsin; Weichselbaum prep. (Kolle and Wassermann.)

Chain formation is common, especially in artificial media, although the chains are usually shorter than those of Streptococcus pyogenes (Fig. 42). Oval and elongated bacillary forms sometimes occur. A well-defined capsule envelops the pneumococci in animal exudates (Fig. 43), but except in certain strains or in certain media is less readily demonstrable in cultures grown outside of the animal body. Capsular substance may often be well demonstrated in milk cultures and in media containing blood or serum. Since the capsular substance is most abundantly developed in recently isolated virulent strains and when the organism is growing in contact with animal tissue and fluids, it has been suggested that the capsule has a protective significance and in some way screens the pneumococcus against

the injurious influences of the body fluids of its host. Infection with Pneumococcus mucosus or Type III pneumococcus (pp. 233, 241), in which capsular substance is especially richly developed, is attended by a particularly high mortality. The pneumococcus is readily stained with the anilin dyes, is gram-positive, asporogenous, and non-motile.

Cultural and Biological Characters.—Some pneumococci grow upon the ordinary culture-media, such as nutrient agar, although never luxuriantly. Fresh meat infusion should be used in preference to beef extract; the hydrogen-ion concentration should be from P_H 7.6 to P_H7.8, and the Arnold method used for sterilization. The addition of sterile defibrinated rabbit blood (3 to 4 drops to 5 c.c. of agar or broth) provides a favorable medium for the growth of the pneumococcus. On this meat-infusion blood-agar the colony is small, moist, translucent and granular, with well-defined edges. A zone of greenish discoloration appears around the colony.

Litmus milk is promptly acidified and often, but not invariably, coagulated.

Inulin fermentation is of some value in differentiating pneumococci from streptococci. As a rule, pneumococci ferment inulin, while streptococci do not. The Hiss serum-water medium is used for this determination.[1] Like most other fermentation reactions produced by bacteria, inulin fermentation by pneumococci is liable to irregularities and variations, and although of high differential value is not to be relied upon alone for absolute identification. An important distinctive character of the pneumococcus serving to distinguish it from Streptococcus pyogenes and other closely allied organisms is its solubility in bile,[2] the active solvent agent in the bile being cholic acid. Fresh ox bile is added in the proportion of about 10 to 20 per cent. to a young, plain broth culture. Insolu-

[1] "Clear beef serum is added to 2 or 3 volumes of distilled water. Heat the mixture for fifteen minutes in an Arnold sterilizer at 100° C. to destroy ferments present in the serum. Add 5 per cent. aqueous litmus solution in a concentration of 1 per cent. or an amount sufficient to give a deep blue color. Add inulin to the serum water in a concentration of 1 per cent. The inulin may be best sterilized by autoclaving at 15 pounds' pressure for fifteen minutes. Sterilize the inulin serum water by the fractional method, 100° C." (Avery, Chickering, Cole, and Dochez: Monograph No. 7, Rockefeller Institute for Medical Research, October 16, 1917, p. 20).

[2] Neufeld: Zeit. f. Hyg., 1900, 34, p. 454.

bility of certain pneumococcus strains has been reported, but is believed to be comparatively rare. The workers in the Hospital of the Rockefeller Institute report that "among several hundred strains of pneumococcus isolated by us from lobar pneumonia none has failed to be dissolved by bile."[1] Pneumococci killed by heat are not bile soluble.

Pneumococci may, therefore, be distinguished from streptococci by their bile solubility and, less definitely, their ability to ferment inulin, greater pathogenicity for mice, and the characteristics of the colonies on blood-agar.

The temperature range of the pneumococcus is narrow, growth taking place, as a rule, only between 25° and 42° C. Cultures of pneumococci in blood broth remain viable for several weeks in the refrigerator. Both the vitality and the virulence of the organism may be conserved for months in the dried spleens of infected mice when these organs, after desiccation in vacuum, are kept sealed in the cold.

Varieties.—As with many other pathogenic bacteria, so with the pneumococci, we are not dealing with a single, uniform, sharply delineated kind of micro-organism, but with a fairly large biological group comprising several types and varieties more or less readily distinguishable one from another. Cultural differences have thus far not given a satisfactory basis for differentiation. Inulin fermentation shows considerable variation and irregularity, and no consistent varietal grouping seems possible on this test alone, although it is probable that in some instances the reported failure of pneumococci to ferment inulin is due to deviation in culture-media and not to the characteristics of the micro-organism.

The most satisfactory means of differentiating varieties of pneumococci is by the use of immunity reactions. This method of differentiation has been applied extensively in the study of pneumonia at the Hospital of the Rockefeller Institute.

Observations by Dochez and Gillespie[2] indicate that the pneumococci found in cases of lobar pneumonia can be divided into four distinct groups ("Types I, II, III, and IV") on the basis of specific agglutination with the serum of immunized animals and

[1] Avery, Chickering, Cole, and Dochez: Monograph No. 7, Rockefeller Institute for Medical Research, October 16, 1917, p. 14.

[2] Dochez and Gillespie: Jour. Amer. Med. Assoc., 1913, 61, p. 727.

protection of animals against infection. In New York City during a period of two years Dochez and Avery[1] found that every strain studied fell into one or another of these four groups. In addition to the constant differences found in immunity experiments with animals, the groups appear to have significant differences in human pathogenicity. The most severe types of pneumonia in man are produced by organisms belonging to the so-called fixed Types I, II, and III. The organisms of these three groups differ from those of Type IV in that their immunity reactions are fairly constant. These groups are never found except in lobar pneumonia; together they are responsible for about 80 per cent. of the cases of this disease. Type IV is composed of a number of strains differing from one another in serologic reactions and rarely found in cases of pneumonia terminating fatally. This heterogeneous group of organisms of low pathogenic power occurs commonly in the normal human mouth.

Type III produces a heavy mucoid growth due to its luxuriant capsule formation and is morphologically similar to Streptococcus mucosus, from which organism, however, it may be distinguished by its bile solubility, ability to split inulin, and greater virulence for mice.

The following table shows the proportionate occurrence of the four types in 454 cases:[2]

Occurrence of Various Types of Pneumococcus in Lobar Pneumonia (454 Cases)

Pneumococcus Type.	Incidence.	Per Cent.
I	151	33.3
II	133	29.3
II (atypical)	19	4.2
III	59	13.0
IV	92	20.3

It is probable that the types here enumerated as occurring in the United States do not include all the differentiable organisms of this group. Lister, in fact, has reported finding at least one additional type in South Africa.

[1] Dochez and Avery: Jour. Exper. Med., 1915, 21, p. 114.

[2] Avery, Chickering, Cole, and Dochez: Monograph 7, Rockefeller Institute for Medical Research, October 16, 1917, p. 18.

Rosenow, in studying transmutations within the streptococcus-pneumococcus group,[1] has come to far-reaching conclusions regarding the possibilities of conversion of one type into another. According to his observations, hemolytic streptococci have been converted into typical pneumococci and vice versâ. Morphology, capsule formation, fermentive power, specific immunity response (opsonin production, agglutination) have all been found by Rosenow to vary within wide limits. The practical significance of these observations is uncertain.

Distribution.—The pneumococcus is a common inhabitant of the human mouth and throat. Pasteur in 1880 recovered from a rabbit inoculated with human saliva a coccus that in all probability was a true pneumococcus. At about the same time Sternberg in this country independently discovered a similar coccus in "a fatal form of septicemia in the rabbit produced by the subcutaneous injection of human saliva." More recent studies have shown that from one-third to one-half of healthy individuals not known to have been in contact with a pneumonia patient habitually carry pneumococci in their mouth secretions.

The results of the examination at the Rockefeller Hospital of the mouth secretions of 297 normal persons showed a great preponderance of pneumococci of Type IV, the most ill-defined and least pathogenic of the four main types recognized in this country. On the other hand, Types I and II, which together cause about two-thirds of all cases of lobar pneumonia, are rarely if ever found in the mouth secretions of normal individuals.

The pneumococcus may survive in external nature for a considerable period. Netter[2] found it in the dust from the walls of a sick-room, and similar observations have been made by other workers. Stillman[3] has reported the interesting observation that pneumococci are more commonly present in the dust of rooms in which cases of lobar pneumonia (Type I and Type II) have occurred than in the dust of rooms in which there has been no lobar pneumonia, and also that in the latter instance pneumococci of Type IV preponderate, as they do in the secretions from the normal mouth.

[1] Rosenow: Jour. Infect. Dis., 1914, 14, p. 1.
[2] Netter: Compt. Rend. de la Soc. Biol., 1897, 49, p. 538.
[3] Stillman: Jour. Exper. Med., 1917, 26, p. 513.

Epidemiology.—It was long a belief that the occurrence of pneumonia was largely, if not altogether, dependent on predisposing influences, such as fatigue and exposure to cold, since observations showed pneumococci usually present in normal mouths. In this view the communication of pneumococcal infection by transfer of the specific germ from one person to another was thought to be of comparatively minor significance and the possibility of contact infection practically disregarded. The differentiation of pneumococcus types and the suggestive facts discovered about the distribution of these types led many workers to a different interpretation.

The types of pneumococci that appear to cause most of the cases of lobar pneumonia (Types I and II) are rarely found in normal individuals without definite history of exposure. The distribution of the various types in the dust of sick-rooms corresponds in some instances very closely with the presence of cases of lobar pneumonia. The following figures illustrate the experience of the Rockefeller Institute in this respect:

	Contacts.		Non-contacts.	
Pneumococcus.	Number Examined.	Percentage Positive.	Number Examined.	Percentage Positive.
Type I	160	13	297	0.3
Type II	149	12	297	0.

This observation indicates the existence of pneumococcus carriers perhaps comparable to the well-recognized carriers of diphtheria and other respiratory tract diseases. Just how important pneumococcus carriers may be in spreading infection is not known. Contact with both cases and carriers in the general population may give rise to new carriers, but very rarely is it possible to trace a case of pneumonia to a pre-existing case. Jacobson[1] found that the incidence of fixed type pneumococci among the attendants in the pneumonia wards of a Chicago hospital was practically the same as that among university students not in contact with pneumonia.

Pneumonia sometimes occurs to a very unusual extent in particular localities and among special groups of people. At one time the pneumonia death-rate among the negro laborers brought to work on the Panama Canal was as high as 18.74 per thousand, and a similarly high incidence of the disease was observed in 1912

[1] Jacobson: Jour. Preventive Med., 1927, 1, p. 259.

among the native laborers in the Rand mines in Africa. Barrack life seems especially to favor the occurrence of pneumonia, and troops in army encampments have often suffered out of all proportion to the same age groups in civil life.

These facts have been variously interpreted—by some as indicating the importance of predisposing factors, such as bad ventilation or fatigue; by others as evidencing the increased opportunities for the dissemination of mouth germs afforded by close personal contact. Since overcrowding in living and sleeping quarters entails both unhygienic conditions of life and better opportunities for the transfer of disease germs, it is not easy to secure evidence that establishes either view to the exclusion of the other. There seems no reason why both factors should not be operative to varying degrees under varying conditions. The segregation of pneumonia patients in order to prevent the spread of pneumonia has theoretically something to recommend it; its actual value is yet undetermined.

Pathogenicity for Man.—The well-nigh universal occurrence of the pneumococcus in the tissue of inflamed lungs in cases of lobar pneumonia, especially in those parts where the pathologic changes are most active, and also its regular appearance in the sputum of the majority of pneumonia patients, afford strong arguments for connecting this organism causally with lobar pneumonia. The same is true of many cases of lobular pneumonia. In many cases the pneumococcus is present in the circulating blood. Observations differ as to the frequency of pneumococcus septicemia, the reported positive cultures from blood ranging from about 20 per cent. to over 90 per cent.; the difference is attributed by some workers to the technic employed. Opinions also differ as to the significance to be attached to the presence of the pneumococcus in the blood-stream. The majority of investigators believe that there is a definite connection between positive blood-cultures and the severity of the disease, and that the presence of the pneumococcus in the blood increases the probability of a fatal termination.

It is sometimes held that lobar pneumonia is caused by bacteria that are inhaled and make their way directly to the alveoli of the lungs (Weichselbaum[1]). Lobular pneumonia, on the other hand, is supposed to be due to the aspiration of bacteria that are present in the bronchial exudate or to a general extension of a bronchitis

[1] Weichselbaum: Kolle and Wassermann, Handbuch, 3, p. 240.

into the alveoli. The type of pneumonia produced by such organisms as Hemophilus influenzæ and B. diphtheriæ is usually of the latter variety (bronchopneumonia). Since these bacteria are probably present in the first instance in the exudate of the upper respiratory passages, the bronchopneumonia may be looked upon as more or less direct extension of the initial infection. A far greater variety of microbes is found associated with lobular pneumonia than with lobar, both as the primary exciting agents and as participants in mixed infections. Probably over 95 per cent. of all cases of pronounced lobar pneumonia are due to the pneumococcus. The so-called atypical cases of lobar pneumonia are also due, in the great majority of cases at any rate, to the pneumococcus, and there is no reason for assuming that a case presenting anomalous symptoms is caused by an unusual bacterial species. The differences observed in pneumococcus infections are related to the susceptibility of the affected individual, and perhaps in some degree to the virulence of the infecting micro-organism. In lobular pneumonia, and more rarely in lobar pneumonia, mixed infections may occur, the pyogenic micrococci being the accessory organisms most commonly found. As already pointed out, both streptococci and staphylococci are capable by themselves of provoking pneumonic processes of the lobular type, and when they are found with the pneumococcus they may be regarded as taking some part in producing the observed pathogenic effects. How great an influence is exerted by such mixed infections upon the outcome of a case is still uncertain. The question as to how far the anatomic and clinical character of a pneumonia is affected by the nature of the microbe or microbes concerned is also unsettled.

The pneumococcus must be held responsible not only for the causation of most cases of both lobar and lobular pneumonia, but for a number of other pathologic processes and conditions. Among the most common of these are inflammations of the pleura, pericardium, endocardium, and meninges. These may occur either as independent and primary affections or as complications and sequels of pneumonia. Inflammations of the meninges, and particularly of the middle ear, are rather frequently secondary to pneumonia; they are sometimes primary infections. The connection between inflammation of the middle ear and meningeal infection has been often noted. There can be no doubt that the pneumococcus on its

road to the cerebrospinal membranes travels occasionally by way of the nasal passages, but the pneumococcus is frequently found in the blood, and other modes of access are also possible.

To these inflammatory conditions of pneumococcal origin might be added a long list of others provoked by the same organism. There appear to be few, if any, organs or tissues that are not under some circumstances subject to attack. Enteritis, conjunctivitis, and a great variety of other affections are occasionally due to the activity of the pneumococcus. In general, pneumococcus infections tend to a more favorable outcome than similar infections with streptococci or staphylococci, but the statement admits of exceptions.

In the production of pneumonia an important part is played by causes affecting individual predisposition. The well-known age-fatality of the disease which bears most heavily upon infants and upon the aged demonstrates the influence of bodily conditions in determining the course of infection. Disturbance of the circulation due to severe or sudden exposure to cold is another familiar factor of causation. The influence of alcoholism as a predisposing factor is especially marked. A variety of other causes, such as infection with a disease like measles or typhoid fever, can lead to a lessening of the normal resistance, so that pneumococci which are taken in with the inspired air, or are perhaps present in some part of the respiratory tract, can penetrate the alveoli of the lungs and excite the pneumonic process. In most, if not all, of the infectious diseases the influence of predisposing factors is important; in pneumonia it is of almost supreme significance.

At the same time the possibility of direct transmission of infection must be kept in mind. It has already been pointed out that pneumococci can retain their vitality in fine spray and in dust for a short time and in dried sputum for a considerably longer period, extending even over some weeks. As in many other bacterial diseases, so also in pneumonia, convalescents and persons coming in contact with patients or convalescents may carry virulent germs in their respiratory tract for weeks or months. Sometimes an apparently simple "cold in the head" may be due to the pneumococcus. Many cases are on record of apparently rather direct communication of pneumonia from one person to another, but few such cases have been studied with adequate bacteriologic methods. It is plain, however, that disinfection of pneumonic sputum should

be rigorously practised, and that the proximity of a pneumonic patient should be avoided, especially by the aged or those in a condition inviting infection.

Pathogenicity for the Lower Animals.—Animal inoculation has thrown a flood of light upon the nature and course of pneumococcus infection in man. The injection of human saliva into rabbits by Pasteur and by Sternberg gave rise to a rapidly fatal generalized infection. The "sputum septicemia" caused by this procedure is now known to be due to the pneumococcus; precisely similar results today follow the injection of pure cultures of this organism. Mice, like rabbits, exhibit a high susceptibility to pneumococcus infection; guinea-pigs are less sensitive. It is a noteworthy fact that in these animals lung lesions, when they occur at all, are slight and usually limited to the bronchopneumonic type. The experiments with the pneumococcus present an example of the general law that susceptibility is characterized by general septicemic infection, resistance by the occurrence of a localized process. Resistant animals, such as the dog, show an approximation toward the type of pneumococcus infection observed in man. It is possible to produce typical lobar pneumonia in the rabbit by carefully balancing the susceptibility of the animal and the virulence of the germ. This may be effected either by employing attenuated cultures of the pneumococcus (injected intratracheally, intravenously, or intraperitoneally), or by partially immunizing the animals so that they acquire sufficient resistance to prevent a general infection.[1]

On the basis of these experiments the course of pneumococcus infection in man may be more readily comprehended. Man must be regarded as an animal of rather high normal resistance. This relative immunity may, however, be so far reduced as to permit of the production of localized manifestations, which in still more susceptible individuals may lead on to a fatal septicemia. In some cases death is due to overwhelming interference with respiration caused by the local pulmonary lesions; in others, to general systemic poisoning or toxemia.

The experimental production of lobar pneumonia in monkeys has been successfully carried out by Blake and Cecil[2] at the Army

[1] Wadsworth: Amer. Jour. Med. Sci., 1904, 127, p. 851.
[2] Blake and Cecil: Jour. Expt. Med., 1920, 31, p. 403.

Medical School, Washington. Pneumococci were introduced into the lumen of the trachea by means of a needle inserted between the cartilages below the larynx. In this way lobar pneumonia showing close parallelism to the disease in man was produced in monkeys with the three fixed types of pneumococci and also with Type IV. Intravenous and subcutaneous injection of pneumococci failed to produce pneumonia.

Pneumococci were found in the blood within six hours after the introduction of the organisms into the trachea and before the signs of pneumonia appeared. These facts favor the view of the bronchogenic rather than the hematogenous origin of the disease. The lesions produced in monkeys were identical in character with those seen in lobar pneumonia in man. Empyema, pericarditis, and other complications occurred in some of the monkeys just as they do in some cases of human pneumonia.

Virulence and Toxin Production; Agglutination; Precipitins.—The occurrence of cases of pneumonia differing widely in severity has been supposed to indicate the existence of strains of pneumococci of varying degrees of virulence, although in any given instance it is plainly impossible to apportion the relative influences of individual susceptibility and of bacterial virulence. The occasional appearance of epidemics of pneumonia of a highly virulent character has also been conjectured to be due to the presence of strains of unusual pathogenic power. These inferences are supported to some extent by the experimental evidence, which shows that considerable fluctuations in virulence occur in cultures. The virulence of a given strain may be greatly exalted by animal passage. This fact has been adduced to explain the virulence of the epidemic type of pneumonia, it being supposed that pathogenicity is heightened by frequent transfer from person to person. Attenuation of pneumococci may also be effected experimentally in various ways, such as growth at a temperature above 39° C. or in a medium in which abundant acid production occurs (milk); in the ordinary culture-media a spontaneous diminution of virulence takes place.

Exact experimental determination of the virulence of a strain of pneumococcus is limited by the fact that virulence for one animal species does not necessarily correspond with virulence for another species. While it is true in general that the virulence for mice of pneumococci isolated from the blood of pneumonia patients is high

and bears some relation to the severity of the symptoms, this is by no means always the case. Perhaps the most definite evidence for the existence of strains of different degrees of virulence is that afforded by the characteristics of the various types already described.

These four types, which are separable by their antibody production, are apparently associated with cases of pneumonia of varying degrees of severity. In Type I infection the mortality is approximately 25 per cent.; in Type II, 32 per cent.; in Type III, 45 per cent., and in Type IV, 16 per cent. It may not be desirable to stress these differences too much until a more extensive series of data is available, but it is not an extreme position to hold that such differences in mortality as that between Type III and Type IV infections marks a real and important difference in virulence between pneumococci also distinguishable in other ways.

Toxin production by the pneumococcus has been the subject of much inconclusive investigation. No soluble toxin secreted by the cell during life has yet been demonstrated. Disintegration of the cell body, however, by freezing and grinding, by the action of bile salts, or by autolysis yields a solution possessing hemolytic and acutely toxic properties. Little is known of the nature of the toxic substances. They may have existed preformed within the body of the cell, as is known to be true of the hemolytic substances and intracellular toxins; or they may be products split off during the disintegration of the bacterial proteins. It is uncertain to what extent the results of pneumococcus infection may be attributed to the action of such substances. Neutralization by antipneumococcus serum is not strictly specific.

Agglutination of pneumococci by the blood-serum of pneumonia patients has been demonstrated by a number of observers.[1] The clumping is accompanied by noticeable changes in the capsular and cell substance, which have impressed some observers as indications of a lytic or degenerative process. It has been shown, however, that although the cocci are profoundly affected morphologically by the agglutinating serum, they retain their vitality for a long time (twenty-five days, Rosenow) and even their virulence. The degree of agglutination produced by the serum of pneumonia

[1] Besançon and Griffon: Ann. de l'Inst. Pasteur, 1900, 14, p. 449; Neufeld: Ztschr. f. Hyg., 1902, 40, p. 54; Wadsworth: Jour. Med. Res., 1903, 10, p. 228; Rosenow: Jour. Infect. Dis., 1904, 1, p. 280.

patients is at best feeble. The highest dilutions at which agglutination is obtained are not, as a rule, over 1:40 or 1:50. The agglutinative property of the blood is most marked about the time of the crisis, and then gradually diminishes.

The agglutinative reaction has been of practical value primarily in identifying the type of pneumococci. The serum of animals immunized with the several types is used preferably in the following dilutions: Type I, 1:40 (*i. e.*, 0.5 c.c. of 1:20 serum to 0.5 c.c. bacterial suspension); Type II, 1:40 and 1:2 (0.5 c.c. undiluted serum to 0.5 c.c. bacterial suspension)—the latter for the purpose of identifying the atypical members of Type II; Type III, 1:10. Specific agglutination of the types occurs in about an hour's time at 37° C. and is observed macroscopically. A bile-soluble coccus not agglutinating with any of the sera enumerated is classed as Type IV pneumococcus.

A specific substance is produced in pneumococcus broth cultures which gives the precipitin reaction (p. 185) when mixed with the homologous antipneumococcus serum. Thus Type I serum gives a specific precipitation with the glucose-blood-broth culture—cleared by centrifugalization—of Type I pneumococcus, but not with that of other types. This fact has been taken advantage of by Avery[1] in devising a rapid cultural method for the determination of pneumococcus types. This method is especially applicable when white mice are not available for the inoculation test. It has given excellent results in the hands of a number of investigators.[2]

Immunity and Serum-therapy.—An attack of pneumonia in man is probably followed by some increase in resisting power, but such acquired immunity is far from permanent. One attack may succeed another after a short interval; in some cases predisposition to a fresh attack seems to be increased. Susceptible animals (rabbits), however, may be rendered immune by inoculation with dead or attenuated pneumococci, followed by inoculation with virulent cultures. The degree of resistance obtained is often considerable.

It is not certainly known upon what this resistance depends. On the basis of animal experiments some observers (*e. g.*, Wassermann[3]) concluded that immunity was due to the production of a

[1] Avery: Jour. Amer. Med. Assoc., 1918, 70, p. 17.
[2] Vaughan, W. T.: Jour. Amer. Med. Assoc., 1918, 70, p. 431.
[3] Wassermann: Deut. med. Wchnschr., 1899, 25, p. 141.

bactericidal substance. Later experiments, however, have shown that pneumococci develop in normal and in pneumococcic human serum with equal rapidity. In fact, neither a bactericidal nor an antitoxic property has been shown to exist in immune serum. Rosenow[1] found that non-virulent pneumococci are susceptible to phagocytosis, while virulent strains are not. The extent to which phagocytosis is important in bringing about the crisis and healing of pneumonia is still undetermined. Clough[2] observed that in a considerable proportion of cases a definite phagocytic activity develops in the serum of the patient at the crisis (or lysis). This increased activity of the serum is such as to bring about active phagocytosis of a virulent pneumococcus not phagocytable in normal human serum. This phagocytic activity seems in practically all cases to be strictly limited to the homologous strain, derived from the patient whose serum is being tested. It is thought probable that the phagocytic factor plays an important part in bringing about recovery in man.

Important results have been reported in serum therapy in connection with the work of Dochez and others (see p. 233) at the Hospital of the Rockefeller Institute. The antiserum developed with pneumococcus cultures of Type III has so low a potency in animal experiments that it has not been used in treating human cases. The antiserum of Type II has given promising results in animal experimentation, but has been disappointing in its effect upon cases of pneumonia in man. The antiserum of Type I, on the other hand, has yielded very encouraging results. The average mortality in cases of this type not treated with serum is 25 to 29 per cent.; the use of the specific serum has reduced the case mortality to less than 5 per cent.

Prophylactic vaccination with killed cultures has been advocated for use under conditions of special exposure, as in army camps. Animal experiments have yielded results that encourage this procedure.

[1] Rosenow: Jour. Infect. Dis., 1906, 3, p. 683.
[2] Clough: Bull. Johns Hopkins Hosp., 1913, 24, p. 295.

CHAPTER XII

THE MENINGOCOCCUS; NEISSERIA (MICROCOCCUS) CATARRHALIS

Genus: Neisseria. Strict parasites, failing to grow or growing very poorly on artificial media. Cells normally in pairs. Gram-negative. Growth fairly abundant on serum media.

A number of closely related organisms have been placed together as a single genus (Neisseria) in recent classifications. The two chief representatives, the meningococcus and the gonococcus, are, as a rule, found in such different lesions and parts of the body that they are not likely to be confounded. Rare cases of meningitis due to the gonococcus have, however, been reported. The serum reactions of immunized animals, while indicating a close relationship of the two organisms, usually permit satisfactory differentiation. The fermentation reactions of the group give further differential data:

	Dextrose.	Maltose.	Saccharose.	Levulose.
Meningococcus	+	+	−	−
Gonococcus	+	−	−	−
N. catarrhalis	−	−	−	−
N. pharyngitidis-sicci	+	+	+	+

Certain gram-negative cocci found frequently in the nasopharynx (Micrococcus flavus I, II and III) are characterized by pigment production and are probably not closely related to the genus Neisseria.

The Meningococcus

Inflammation of the meninges or investing membranes (pia-arachnoid) of the brain and spinal cord may be provoked by a variety of organisms, and may occur either as a primary affection or secondarily in the train of an infection originally begun elsewhere. One form of meningitis, characterized especially by epidemic spread and usually designated as *epidemic meningitis*, spotted fever, or cerebrospinal fever, is accompanied by the presence of a specific micro-organism, commonly known as the meningococcus.

This organism, to which the name Neisseria (Micrococcus) meningitidis or N. intracellularis has been given, appears to have been seen by Leichtenstern in the meningeal exudate as early as 1885. The first important work upon it, however, was that of Weichselbaum,[1] who in 1887 described it in detail as the characteristic micrococcus found in 6 cases of acute cerebrospinal meningitis. Weichselbaum also carried out successful animal experiments. Some years later the conclusions arrived at by Jäger[2] in connection with an outbreak of epidemic meningitis in the military garrison at Stuttgart, although based to some extent on faulty observations, corroborated Weichselbaum's results in their essential features, and were the cause of renewed interest in this subject. The etiologic rôle of the meningococcus has since been securely established by a number of investigations, among which may be mentioned especially the extended researches of Councilman, Mallory, and Wright.[3] An admirable recent study of the disease has been made by Foster and Gaskell.[4]

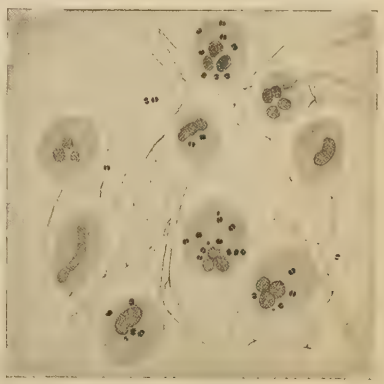


Fig. 44.—Meningococcus in pus cells (Councilman): Pus cells containing diplococci from the meninges. A few diplococci are in the exudate outside of the pus cells. Between the pus cells there are delicate fibrillæ of fibrin. The illustration is an accurate representation of a group of cells in the field of the microscope.

Morphology.—In cover-slip preparations of the meningeal exudate the meningococcus appears in diplococcus or in tetrad form (Figs. 44 and 45). The micro-organism occurs characteristically in the interior of the polymorphonuclear leukocytes, these cells being sometimes so packed with diplococci that the nucleus is obscured. When tested by Gram's method of staining, decolorizing takes place. This character serves to distinguish it readily from the ordinary streptococci and from the pneumococcus. In cultures the meningococcus is about 1 μ in diameter, and appears,

[1] Weichselbaum: Fortschr. d. Med., 1887, 5, p. 573.
[2] Jäger: Ztschr. f. Hyg., 1895, 19, p. 351.
[3] Special Report of the State Board of Health of Massachusetts, 1898.
[4] Foster and Gaskell: "Cerebrospinal Fever," Cambridge, England, 1916.

as a rule, in pairs; short chains are seen more rarely. No capsule is present, although irregularities in staining and the occurrence of swollen cells have led to some confusion on this point. Involution forms are common.

Cultural Characters.—The most useful medium for growing the meningococcus is blood-agar, prepared by adding 1 c.c. of fresh defibrinated or laked blood to 10 c.c. of ordinary nutrient agar (P_H 7.4–7.6). The blood of the sheep, goat, rabbit, horse, or man can be used. On this medium the meningococcus

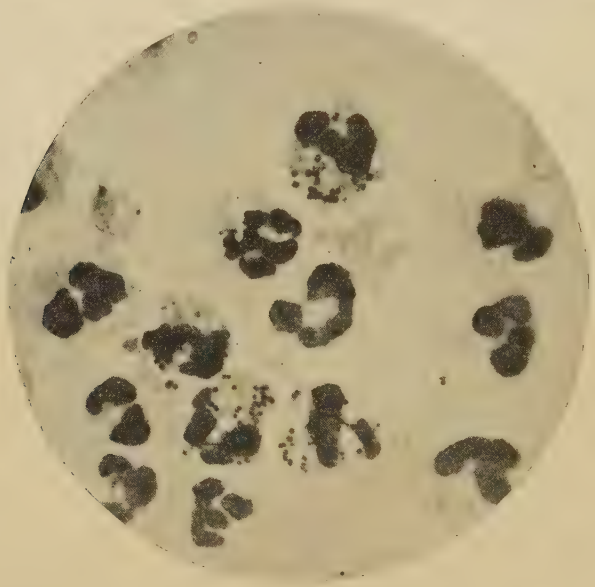


Fig. 45.—Meningococci in pus from brain abscess (Flexner).

colonies are moist, elevated, smooth, and with a bluish-gray tinge. They do not produce green coloration or hemolysin, and can be readily differentiated by their relatively large size and appearance from the hemolytic streptococcus and the green streptococcus (Streptococcus viridans) and from the pneumococcus, but resemble closely the colonies of Micrococcus catarrhalis (p. 255). They are not so whitish or opaque as the colonies of staphylococcus. In isolating the meningococcus from the throat of a carrier it is essential that the culture-medium be warm when inoculated and be kept warm until finally placed in the incubator. On blood-agar the meningococcus commonly dies out within a few

days, but vitality may be preserved for several weeks in stab-cultures in starch-agar (1 per cent. cornstarch added to ordinary nutrient agar).

The fermentation reactions of the meningococcus give valuable aid in differentiating it from closely allied organisms. Its ability to ferment maltose distinguishes it from the gonococcus, as already pointed out (p. 244).

Other related cocci, such as N. pharyngitidis-sicci and several varieties of M. flavus, ferment various carbohydrates, such as mannose, saccharose, and levulose, not attacked by the meningococcus.

Antigenic Varieties.—The meningococci, while culturally alike, are not a simple homogeneous group in their immunological relations. In 1909 Dopter[1] reported the discovery of a "para-meningococcus" which, although isolated from typical cases of cerebrospinal fever and possessing the usual cultural characters of the "regular" or "normal" meningococcus, failed to agglutinate with antimeningococcal serum. Furthermore, while these cases were not helped by the administration of antimeningococcal serum, they were successfully treated by injection of serum prepared with the parameningococcus.

Gordon[2] has further separated the meningococci into four serological groups. His findings have been confirmed by Tulloch, who reports that practically all of the meningococci isolated from 354 cases of cerebrospinal fever fall into one or another of Gordon's four groups.[3]

The existence and relative frequency of different antigenic strains of meningococci is a matter of practical importance. It has happened more than once that sera prepared by using certain strains has proved ineffective when administered to meningitis cases that chanced to be caused by other strains. The present custom of preparing a polyvalent serum, obtained by injecting horses with a number of different meningococcus strains, has therefore a sound experimental basis.

Epidemiology.—As in most of the respiratory tract infections, mouth spray from persons infected with meningococci is an important agent in spreading the disease. The relative infrequency of

[1] Dopter: Compt. rend. Soc. biol., 1909, 67, p. 74.
[2] Gordon: Jour. Hyg., 1918, 17, p. 290.
[3] Tulloch: Jour. Hyg., 1918, 17, p. 316.

infection among doctors, nurses, and others in direct contact with meningococcus patients shows, however, that individual susceptibility of adults to this infection is not very common. Cases of direct contagion have been described, but are not numerous. The comparative rareness of meningitis throughout the adult civilian population is another indication that human susceptibility is low. The influence of predisposing causes in increasing susceptibility is thought to be especially marked under conditions of military life where meningitis often prevails excessively among troops in barracks and tents. Insufficient clothing, inadequate ventilation, exposure to inclement weather, and perhaps fatigue are believed to play a part in reducing the normal resistance of the new recruit. Along with such influences it is necessary to consider the greater opportunities for the spread of the disease by meningococcus carriers in camp life. The close contact in sleeping quarters necessarily favors the spread of all mouth spray infections. Observation has shown that at times when meningitis is prevalent among troops healthy meningococcus carriers are especially numerous. It seems to be true, therefore, that under the conditions of military life in recruiting camps a good many individuals acquire meningococci from their associates; a certain proportion of these, owing to individual peculiarities or to the action of predisposing causes, develop into "cases" of meningitis, while the majority become free in a short time from the meningococci that have found temporary lodgment in their nasopharynx.

The share of the meningococcus carriers in the transmission of infection is generally considered of great importance. Meningitis patients and convalescents and healthy persons who have simply picked up the germs from others may be the means of conveying infection. As in other infections, meningococcus carriers are often divided into permanent carriers and temporary carriers according to the length of time that they harbor the germs; the distinction is naturally more or less arbitrary.

It is believed that next to the removal or avoidance of predisposing factors, the discovery and control of permanent meningococcus carriers offers the most promising method for diminishing the spread of meningitis in military organizations.

The following excerpts from a memorandum agreed upon by representatives of medical departments of the United States Army,

Navy, and Public Health Service give a basis for a standardized technic for the detection of meningococcus carriers.

STANDARD TECHNIC OF MENINGOCOCCUS CARRIER DETECTION

I. CULTURING.

a. Wherever possible the cultures should be taken in a small room in the regimental infirmary. The floor of this room should be washed with an abundance of soap and water one hour before the men enter for culture, and should be wet during the process of culturing.

b. Only a *few men* at a time should be admitted to the culture room.

c. Windows and doors should be closed all the time.

d. Cultures should not be made within an hour after meals.

e. No men should be examined on the same day on which they are sprayed.

II. METHOD OF SWABBING.

a. The swab used should be:

1. A naked wire 25 cm. long, with a small absorbent cotton pledget on one end, well covering the end of the wire, and a ring handle on the other. The wire should be flexible, such as stove pipe wire or hay baling wire, of about 18 gauge. The swabs may be sterilized in glass or paper containers in groups of 5–20. The last one or two centimeters of the pledget end of the swab is bent to an angle of about 30–40 degrees. This swab is simple and has proved most satisfactory, and can be at once discarded.

2. West Tube. This gives satisfactory cultures, but is cumbersome, and not strictly necessary.

3. Straight unprotected nasal swab. This is useful in the case of individuals with highly irritable throats, but is not recommended as a routine.

b. The subject should be seated facing the light. Tongue depressors are to be used when necessary. The swab should be passed behind the soft palate while the subject is phonating. The swab, having passed up behind the palate, is introduced successively into each posterior naris, and then is drawn across the posterior wall of the nasopharynx. The swab is then withdrawn, taking care not to touch the throat surfaces or the tongue; this is best accomplished during phonation.

The success of carrier search depends largely on the care with which the swabbing is done, hence the man taking the cultures should be able to execute these directions skilfully. He should be either a nose and throat specialist, the bacteriologist himself, or directly under the latter's supervision.

III. METHOD OF INOCULATING PLATES.

a. The mucus-charged swab should be applied over a limited area at the periphery of the plate. From this the spread is made by a wire loop passed by a series of radial strokes, each starting from the infected point. If there is very little mucus it is often possible to smear directly from the end of the swab.

b. A single person per 10 cm. plate is preferable. More than two cultures on one plate should never be made.

c. The plate must be inoculated while warm and kept warm until replaced in the incubator. To accomplish this sterile plates already warmed by storage in the incubator should be packed in a device insulated against the loss of heat, such as a fireless cooker, for transportation.

Care should be taken to keep the plates warm during the inoculation process. It is advisable to carry a plate seeded with known culture of meningococcus to act as control.

V. EXAMINATION OF INOCULATED PLATES.

a. Plates should be inverted when placed in the incubator.

b. Plates will be ready for examination after twelve to eighteen hours' incubation at 37.5° C.

c. Discard all plates that are crowded and do not show discrete, well separated colonies.

d. The meningococcus colony on the whole blood medium does not produce green coloration or hemolysis. It tends to be somewhat larger than the streptococcus and pneumococcus. The colonies are moist, elevated, outlines are ill defined, and on moderately opaque blood-agar have a faintly bluish tint. The colonies are not usually opaque, a characteristic which distinguishes them from the staphylococcus.

On transparent blood medium and with transmitted light under lens magnification the colony may be nearly clear, but often shows a very faint smoky, gray-blue quality. This characteristic is intensified by passing the finger between the light and the colony to shade it. Further lens effect may be seen by moving the plate so that the colony passes across some distant obstruction to the light as a string stretched across the window. The colony is never granular in young cultures. The colony should be confirmed by smear and gram stain.

VI. TRANSPLANTATION.

The suspected (ringed) colonies are transferred to warmed moist blood-agar slants. They must have been incubated to insure sterility. It is best to keep the tubes continuously in a warm water-bath until finally placed in the incubator. If transfers are made under these conditions there will be enough growth for identification in about eight hours.

Two essentials to success in growing meningococci are keeping the media moist and keeping it constantly from the time of inoculation at body temperature.

VII. IDENTIFICATION.

a. Microscopic. Smear preparations are made in the usual way stained by Gram. The presence of a few gram-positive or other contaminating organisms does not make the culture unsuitable for agglutination.

b. Agglutination. All gram-negative micrococci are subjected to agglutination in the following way:

The serum dilutions are first set up, and the bacterial emulsion is made directly in them. To make this emulsion a loopful of the suspected culture is scraped off, and the loaded wire is passed well down the tube almost to the level of the fluid. The bacterial mass is then

rubbed off against the wall of the tube and mixed with the fluid. In the case of the meningococcus a smooth emulsion is usually rapidly produced. Many of the other organisms are much less readily emulsified.

An emulsion must be free of clumps for proper agglutination tests.

With the polyvalent agglutinating serum supplied by the Army Medical School, Washington, D. C., or the U. S. A. Laboratory No. 1, France, The Naval Medical School, Washington, D. C., or the Hygienic Laboratory, Washington, D. C., agglutinations should be made in a 1 : 100 dilution.

The quantity in each tube should be 1 c.c. The tubes are then incubated at 55 degrees for twelve to eighteen hours, with necessary precautions against evaporation.

Controls. Each culture should be run in parallel with a normal horse-serum control at 1–50. A known meningococcus culture should be run as a positive control with each set.

VIII. READING OF AGGLUTINATIONS.

The tubes which have been clarified are then shaken gently, when the clumps can be clearly detected by the naked eye or hand lens.

The organisms which are agglutinated by the polyvalent serum dilutions at 1 : 100 are to be regarded as meningococci.

Organisms which agglutinate in both polyvalent and normal horse-sera are to be thrown out.

A slight opalescence in the supernatant fluid due to contaminating organisms, if there is otherwise distinct evidence of agglutination, need not lead to the discard of the culture as negative.

IX. TYPING.

The meningococcus isolated by agglutination with polyvalent serum should be typed as soon as possible. If this procedure cannot be carried out on the spot, the cultures, or heated suspensions of the cultures in saline with 0.5 per cent. phenol, should be sent to the nearest Department laboratory.

Diagnostic type sera should be obtained from the Army Medical School, Washington, D. C., or from U. S. A. Laboratory No. 1, France, Naval Medical School, Washington, D. C., or the Hygienic Laboratory, Washington, D. C.

X. CULTURES.

From Spinal Fluid:

Fresh spinal fluid is to be divided into two portions. One part is centrifuged and the sediment smeared, examined microscopically, and planted on blood-agar slants or plates and also in serum-dextrose-broth. The other uncentrifuged portion of the fluid is added directly to serum-dextrose-broth.

The examination of cultures is made at twenty-four-hour intervals for five days. It should be noted that often in the absence of colonies on the surface of the slant there may be a luxuriant growth in the water of condensation.

From Blood:

Meningitis suspects, like all other febrile cases, should be cultured as promptly as possible. About 5 to 10 c.c. of blood, drawn from a

vein by a syringe, should be planted in 100 c.c. of warm dextrose broth. The cultures should be examined at twenty-four-hour intervals over a period of not less than six to eight days. To get the culture going it is necessary to transplant each day for the first five days.

XI. TYPING OF ORGANISMS.

Organisms recovered from spinal fluid or blood should be typed and compared with the organism isolated from the patient's nasopharynx.

Inagglutinable strains are sometimes encountered. To establish further the nature of these organisms they should be grown on dextrose, maltose, and saccharose serum-litmus-agar.

XII. INDICATIONS FOR CULTURING.

On the appearance of a case of cerebrospinal fever all contacts should be cultured for the detection of carriers as soon as possible. By "contacts" is meant those intimately associated with the patient—that is, all those in the same tent or squad room with him, as well as his close associates at mess or elsewhere.

Experience has shown the inadvisability of attempting to culture larger groups than this.

In military service those who give positive cultures should be held in the detention camp until they have had three successive negative cultures at five-day intervals. If a second case appears in the same company within a week of the first case, the whole organization should be swabbed.

XIII. DISPOSAL OF CARRIERS.

The ordinary carrier usually clears up in a week or two.

Those who carry longer than this constitute the chronic carriers, and usually give large numbers of colonies or even pure cultures; these are probably the important cases from a public health point of view.

Such carriers should not be recommended for furlough to their homes, nor discharged from the Service without authority from the Surgeon General.

Pathogenicity for Man.—Many cases of meningitis due to the meningococcus are characterized by special features which distinguish them from cases of meningitis produced by other organisms, as, for example, from pneumococcal meningitis. The most marked lesions occur at the base of the brain, extending from the optic commissure backward over the crura, pons, and medulla. The meninges of the entire brain are rarely affected. The cord is always affected, and to a greater extent than in any of the other forms of meningitis. The usual history is that of sudden onset, and many so-called fulminating cases occur. The disease is most frequent in children and young adults, cases being rare in persons over thirty-five years of age. In the epidemic studied by Councilman, Mallory,

and Wright[1], the mortality was 76 in 111 cases (68 per cent.); in New York city in 1905, out of 2755 cases reported, there were 2026 deaths (73 per cent.). While the mortality is thus usually high, epidemic meningitis stands in contrast to the practically invariably fatal meningitis caused by other bacteria.

During life the surest method of diagnosis of this form of meningeal disease is the detection of the specific micro-organism in spinal fluids obtained by means of lumbar puncture. Elser and Huntoon[2] were able in this way to demonstrate the meningococcus by microscopic examination in 141 out of 171 cases. When cultural methods are also used the proportion of positive findings is increased. Especially prominent among the symptoms are the involvement of the eye and ear, the infection showing a definite tendency to extend along the optic, auditory, and fifth nerves. The meningococcus is sometimes found in the circulating blood. According to some observers the blood infection is primary and the invasion of the meninges secondary. Under some circumstances the meningococcus can give rise to a focal pneumonia, which differs from that produced by the pneumococcus. Complications in general are not very frequent, acute rhinitis being the one most commonly reported. It is still an open question whether the nasal cavity is the usual portal of entry for this organism, and the meningitis secondary to an acute rhinitis, or whether the rhinitis is secondary to a blood infection. In the first days of the disease the meningococcus can be demonstrated in the nasal secretions in a large proportion of cases.

Pathogenicity for Other Animals.—Rabbits and adult guinea-pigs display little susceptibility to inoculation. White mice are somewhat more susceptible, especially to intraperitoneal inoculation. Young guinea-pigs (175 to 200 grams) are quite highly susceptible to intraperitoneal inoculation, but in order to produce a fatal result rather large amounts of culture must be used, and, as a rule, the meningococcus fails to invade the tissues. The guinea-pig experiments indicate that the death of these animals is caused by a poison liberated by the disintegration of the bacterial cells. Cultures killed by heat and cultures subjected to autolysis are quite toxic. Councilman and his co-workers[3] produced typical

[1] Councilman, Mallory, and Wright: Special Report Mass. State Board of Health, 1898.

[2] Elser and Huntoon: Jour. Med. Res., 1909, 15, p. 377.

[3] Special Report Mass. State Board of Health, 1898, p. 77.

meningitis in a goat by intraspinal injection of pure cultures. Flexner[1] has reproduced in monkeys the lesions and to some extent the symptoms of acute meningitis as they occur in man.

Agglutination and Immunity; Curative Antiserum.—Agglutinins are produced by animal inoculation with the meningococcus, and appear also in the blood of patients suffering from epidemic meningitis; the serum of the latter agglutinates the specific cocci in a dilution of 1 : 50 or higher. Kolle and Wassermann[2] have shown that when large quantities of meningococci are injected into the body of a horse, agglutinins, opsonins, and also specific immune bodies (amboceptors) are produced, and that the horse-serum has a curative effect; and Flexner[3] has likewise found that guinea-pigs and monkeys can be saved from otherwise fatal meningococcus infection by a specific antiserum. A curative serum has been prepared by Flexner and Jobling[4] by injecting a horse first with gradually increasing doses of dead meningococci, then of living cocci, and finally of an autolysate. The injection of the anti-meningitis serum directly into the spinal canal in human cases has a marked influence upon the course of the disease. The effect seems to be due partly to an antitoxic action, partly to the stimulus that the serum gives to increased phagocytic digestion, and partly to its direct injurious action upon the cocci. Large doses of the serum are commonly given, 15 to 30 c.c. for children, and about twice as much for adults. Repeated injections (at least three or four at twenty-four-hour intervals) give the best results. Observations of the cerebrospinal fluid after injection show a remarkable destruction of the meningococci. The serum is entirely without effect when introduced directly or indirectly into the blood, and must in all cases be injected into the seat of the disease by lumbar puncture. Of 1294 cases of epidemic meningitis treated with the Flexner-Jobling serum, 894 recovered and 400 died.[5] The average mortality in the pandemic of this disease which began in 1904 and was not wholly at an end in 1913, was about 70 per cent. The mortality in the serum-treated cases just referred to was not quite 31 per cent.; 199 cases injected with serum between the first and

[1] Flexner: Jour. Exper. Med., 1907, 9, p. 142.
[2] Kolle and Wassermann: Deut. med. Wchnschr., 1906, 32, p. 609.
[3] Flexner: Jour. Exper. Med., 1907, 9, p. 168.
[4] Flexner and Jobling: Ibid., 1908, 10, p. 141.
[5] Flexner: Jour. Exper. Med., 1913, 17, p. 553.

third day of the disease showed a mortality of 18 per cent. Complications and sequelæ of the infection are reduced in number in the serum-treated cases.

The use of a polyvalent serum, produced by injection of a number of different strains of meningococci—regular, para and intermediate—seems highly important. Good results have been obtained with the Rockefeller Institute serum prepared with more than 50 strains. Experience does not favor the general use of a serum made with a single strain or with only three or four strains.

N. Catarrhalis

This gram-negative coccus is found quite commonly in the nasopharynx of healthy individuals as well as in persons suffering from colds and other respiratory infections (Fig. 46). The cells as a rule are somewhat smaller than those of the meningococcus. Growth occurs on ordinary nutrient agar much more readily than does that of the meningococcus. Dextrose is not fermented. Different strains vary in their degree of pathogenicity for animals, but many strains are fully as pathogenic as meningococci for white mice and guinea-pigs. In man they appear at times to excite catarrhal inflammation and sometimes pneumonia.

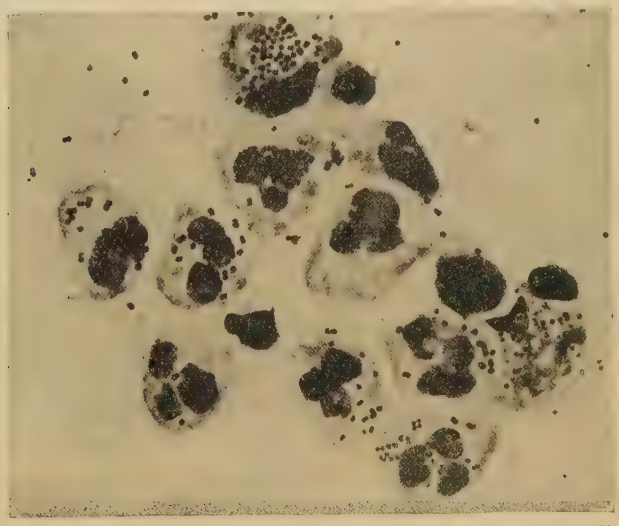


Fig. 46.—N. catarrhalis in smear from sputum (F. T. Lord).

They seem to have been conspicuous secondary invaders in the 1918 influenza epidemic in some localities.

N. Pharyngitidis-sicci

This small gram-negative coccus found on the mucous membrane of the respiratory tract grows at room temperature as well as at 37° C., forms white, firm, dry, adherent colonies, and ferments saccharose, lactose, and mannitose. Although not ordinarily regarded as pathogenic, it has been found as apparently the causal agent in a case of kidney infection.[1]

[1] Kretschmer and Hufnagel: Jour. Amer. Med. Assoc., 1924, 82, p. 1850.

CHAPTER XIII

THE GONOCOCCUS (NEISSERIA[1] GONORRHŒÆ)

Genus: Neisseria. Strict parasite, not growing on ordinary media. Cells in pairs resembling coffee-beans; surrounded by a capsule. Found within pus cells. Gram-negative. Fermentive powers high.

Few diseases are so widely disseminated through all classes of society as gonorrhea. The conservative statistics of Erb,[2] based on the history of about two thousand male patients, mostly among the German middle classes, showed that about 48.5 per cent. had had gonorrhea. Some German authorities would place the proportion in the whole population at a much higher figure. Cabot,[3] in Boston, found that 35 per cent. of a hospital population of 8000 gave a history of gonorrhea.

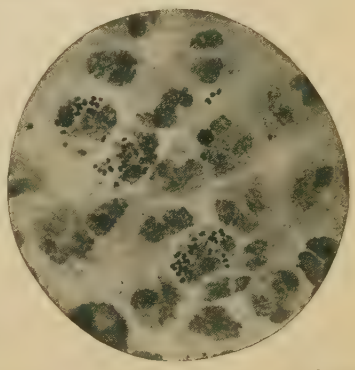


Fig. 47.—Smear from gonorrheal pus. Romanowsky; × 1100 (Hicks).

Neisser[4] in 1879 first called attention to the constant presence of a peculiar coccus in gonorrheal pus. In cases of gonorrhea of recent origin this was the sole organism found; it occurred not only in the urethral and vaginal discharges of ordinary gonorrhea, but was present in the exudations of conjunctivitis due to gonorrheal infection. Pure cultures of this organism were first obtained by Bumm[5] (1885), who also succeeded by inoculation experiments in demonstrating beyond doubt its causal relation to gonorrhea.

Morphologic and Cultural Characters.—Morphologically the gonococcus (Neisseria gonorrhœæ) is very similar to the meningo-

[1] Neisseria is probably the proper generic name for the gonococcus, according to the law of priority in nomenclature.

[2] Erb: Münch. med. Wchnschr., 1906, 53, p. 2329.

[3] R. C. Cabot: Boston Med. and Surg. Jour., 1911, 165, p. 155.

[4] Neisser: Centralbl. f. d. med. Wissensch., 1879, 17, p. 497.

[5] Bumm: "Der Mikroorganismus der gonorrhoischen Schleimhauterkrankungen," Wiesbaden, 1885.

coccus. In preparations made from gonorrheal pus the cells occur in pairs, with the flattened sides in juxtaposition; the appearance in stained preparations is like that of a coffee-bean. Pappenheim's stain (p. 52) is well adapted for determining the presence of the gonococcus in suspected pus. In pure cultures the cocci are often aggregated in irregular masses, but, especially in young cultures, also show the characteristic diplococcus arrangement. Like the meningococcus, *it is found within the pus cells*, sometimes in enormous numbers (Fig. 47). In the earliest stages of infection, however, gonococci are found free in the serum, and the same is true of cases of gonorrhea of long standing.

The cocci in the pus cells do not invade the nucleus, but are confined to the cytoplasm of the cell. There is satisfactory evidence that the gonococci are picked up by the phagocytes and do not actively penetrate the latter; they are, indeed, entirely non-motile. The gonococci found inside the leukocytes are but slightly altered in appearance, and in many cases are still alive; no multiplication, however, has been demonstrated, although it is possible that this occurs. Whether the extensive phagocytosis that takes place has any influence upon the course of gonorrhea is unknown. *When treated by Gram's method, the gonococcus completely loses the stain.*[1] Its behavior toward the gram stain, together with its coffee-bean form and intracellular situation, usually serves to distinguish the gonococcus from related organisms, such as the common pyogenic cocci found in the urethral or vulvovaginal tracts. Compared with the meningococcus, however, the gonococcus grows even less readily on special artificial culture-media, such as blood-agar and serum-agar. Some strains, however, are said to grow on glucose or glycerol-agar from the start, but, as a rule, less luxuriantly than the meningococcus. In practice little confusion is caused by the close resemblance of these two organisms, since they are not liable to occur in the same tissues. Meningitis is, however, in rare instances, caused by the gonococcus. Organisms other than the gonococcus, staining like it and contained in the polymorphonuclear leukocytes, are occasionally, although rarely, found in the urethral and vaginal secretions.

[1] The gonococcus is decolorized rather slowly. In testing for this organism the film should be exposed for ten minutes, instead of five, to the action of alcohol.

Growth seldom takes place upon the ordinary gelatin and agar culture-media unless considerable quantities of pus are smeared on the surface. Bumm was the first to succeed in cultivating the gonococcus, accomplishing this by use of a mixture of ox, sheep, and human serum. Later (1891) Wertheim[1] devised a medium which has proved highly serviceable, and is today, with certain modifications, in general use. The most successful results in every way are obtained when blood or blood-serum is added to the usual nutrient media. Fresh material is much more likely to yield positive cultures than material that has been kept for a time.

Wertheim's serum-agar is prepared by adding from two to three parts of nutrient agar to one part of human serum (or other serous fluid, such as ascitic or hydrocele fluid). The serum is first warmed to 40° C., and then added to liquid agar of the same temperature. After a number of generations upon Wertheim's medium a scanty growth may occur on ordinary agar unmixed with serum. Nutrient agar smeared with sterile human blood is also an excellent medium for the gonococcus. Upon Wertheim's serum-agar the colonies formed by the gonococcus resemble streptococcus colonies, but are in general somewhat larger, less translucent, and of a more tenacious consistency. In ascitic broth (three parts meat broth to one of ascitic fluid) a finely granular growth occurs on the walls and bottom of the test-tube. Toward external influences the gonococcus displays a high degree of sensitiveness. According to most experimenters, cultures are injuriously affected by a temperature of 40° to 41° C. The gonococcus is sensitive to drying, and under ordinary conditions can survive exposure to the air for only a very short time, although in masses of dried pus it may live exceptionally for six or seven weeks. In favorable culture-media it rarely maintains its vitality more than forty-eight to seventy-two hours at room temperature, but, like many other bacteria, will live longer if kept in a refrigerator.

Correct diagnosis of gonococcus infection, based on the discovery of gram-negative diplococci within pus-cells, is often a relatively simple matter, but it must be remembered that there are several sources of error. As already pointed out, gonococci sometimes lie free in the serum and are not contained within the leukocytes. Again, as already mentioned, the occasional presence of organisms

[1] Wertheim: Deut. med. Wchnschr., 1891, 17, p. 1351.

similar to the gonococcus, especially in the vulvovaginal tract, makes it advisable in doubtful cases to fall back on cultural methods. If microscopic findings are negative, a much higher degree of certainty can be obtained by suitable cultural methods in the hands of an experienced observer. Centrifugalization of the urine is often resorted to, and increases the chances of finding gonococci in the sediment either microscopically or culturally.

Inoculation Experiments.—The lower animals (including apes) are not susceptible to inoculation either with pure cultures of the gonococcus or with gonorrheal pus. Whether introduced into the peritoneum or the urethra or applied to the conjunctiva of these animals, the gonococci have shown themselves impotent to effect an invasion of the tissues. The poisonous products contained in cultures have, however, some effect upon animals, and will cause the death of guinea-pigs and white mice. These poisonous substances are not true toxins and are not diffused into the surrounding media during the life of the cell, but, according to the statement of most investigators, are constituents of the bacterial cell-body. On the death of the cell they may appear as disintegration products in the surrounding fluid. They are quite resistant to heat, and are able, according to some investigators, to withstand a temperature of 80° C. or even 115° C. De Christmas[1] and his co-workers reported successful immunization experiments with these toxic substances, but their results have not been corroborated by other experimenters. Inoculation of the human subject (both sexes) with pure cultures of the gonococcus gives rise to a typical infection (Bumm, 1885). The mucous surfaces seem to be especially susceptible, and inoculation of the urethra almost invariably succeeds. Repeated demonstration of the specificity of this organism for gonorrhea has thus been obtained. The poisonous bodies above mentioned will evoke suppuration when injected into the urethra, but since the products of other microbes, as, for example, staphylococci and colon bacilli, produce the same result, the effect cannot be regarded as specific. Little if any immunity is conferred by an infection, reinoculation being successful at short intervals; clinically it is observed that an acute attack may be superimposed upon a chronic gonorrhea. An antibody (amboceptor) has, however,

[1] De Christmas: Ann. de l'Inst. Past., 1900, 14, p. 331.

been found in the blood of gonorrheal patients.[1] Torrey[2] and others have observed that agglutinins and precipitins are produced in rabbits and other laboratory animals inoculated with cultures of gonococci. The close relationship of the gonococcus to the meningococcus is shown with especial clearness in the observation of Martha Wollstein[3] that the agglutinins, the protective power, and the amboceptors developed in the serum of immunized animals seem to be largely common to both micro-organisms.

Results of Gonococcus Infection.—As a rule, the gonococcus attacks primarily the human urethra, and then gives rise to an inflammation which may be followed by chronic urethritis and stricture. The dangerous extragenital complications and sequelæ of this affection are not so generally known as they should be. So far from being a localized inflammation confined to the immediate neighborhood of the original point of infection, the gonorrheal process may be far-reaching in its effects. In the female particularly, the entire genito-urinary tract may be involved, the Fallopian tubes, the ovaries and the peritoneum being invaded not at all uncommonly. Spread of the infection along contiguous mucous surfaces may likewise occur in the male, causing epididymitis and other inflammatory conditions. The gonorrheal ophthalmia of the new-born due to infection at birth is a well-known consequence of maternal infection. Although exact information is not obtainable, it is estimated that 10 per cent. of all cases of blindness are traceable to this source, and that in the United States there are at least 12,000 children blind from this cause.

The gonococcus may also invade the blood from local lesions and be carried by the lymph and blood-streams to distant parts of the body. In this way it can incite a variety of extragenital lesions, some of which result fatally. Especial predilection is shown for the synovial membranes of the joints, where it causes the so-called gonorrheal rheumatism, and for the heart-valves, where it produces endocarditis. It is possible to obtain the gonococcus in pure culture from the affected region in a considerable proportion of cases. According to the statistics of Neisser,[4] gonorrheal metastases occur in about 0.7 per cent. of all cases of gonorrhea

[1] Bruck: Deut. med. Wchnschr., 1906, 70, p. 1368.
[2] Torrey: Jour. Med. Res., 1907, 16, p. 329.
[3] Wollstein, Martha: Jour. Exper. Med., 1907, 9, p. 588.
[4] Neisser: Kolle and Wassermann, Handbuch, 3, p. 182.

coming to the knowledge of physicians. Local or general complications, however, occur in about 30 per cent. of all cases. Metastatic conjunctivitis without direct inoculation of the conjunctiva has been reported. It is uncertain whether this is always due to the presence of gonococci carried to the conjunctival sac in the body-fluids or whether circulating toxic substances act on the cellular elements of the conjunctiva.

A peculiarly dangerous feature of gonococcus infection is the long period during which an infected man or woman may be capable of infecting others. Gonococci may persist in the genito-urinary secretions for years after apparently complete recovery has taken place. By this means serious inflammations of the genital tract are produced in thousands of innocent wives by their previously infected husbands.

Epidemics of gonorrheal vulvovaginitis in little girls due to carelessness in the use of towels, wash-cloths, thermometers and bath-tubs are not uncommon, and are a serious problem in many institutions. Such infection may be followed by the same grave consequences to the female reproductive organs as gonococcus infection produced in any other manner.

Gonococcus Vaccine.—Cole and Meakins[1] have employed Wright's method of subcutaneous vaccination in the treatment of gonorrheal arthritis. An emulsion of gonococci in 0.85 per cent. salt solution heated for one hour at 65° is first injected, the initial dose containing usually about 300 million dead gonococci. The succeeding vaccinations are gradually increased in amount until 1000 to 1200 million gonococci are administered in a single dose. The results obtained by Cole and Meakins were often favorable, and were more marked in the chronic cases than in the acute ones. While the authors express skepticism as to the value of the opsonic index as a guide to the administration of vaccine, and "do not feel that the danger of cumulative negative phases is a real one," they conclude as follows: "In no case have we seen the administration of gonococcus vaccine do harm, and we feel that these cases offer sufficient justification for the treatment of gonorrheal arthritis by means of vaccines in doses of 500 to 1000 million, administered every seven to ten days." Irons,[2] studying a larger

[1] Cole and Meakins: Bull. Johns Hopkins Hosp., 1907, 18, p. 223.
[2] Irons: Jour. Infect. Dis., 1908, 5, p. 279.

series of cases, has also obtained results indicating that in certain cases of gonococcus arthritis recovery can be hastened by the injection of dead gonococci.

Irons[1] has further found that the cutaneous inoculation of glycerol extracts of autolyzed gonococci produces a well-defined reaction in patients infected by the gonococcus. This cutaneous reaction is not usually obtained in normal persons nor in those suffering from other infectious diseases.

[1] Irons: Jour. Infect. Dis., 1912, 11, p. 77.

CHAPTER XIV

THE ANTHRAX BACILLUS

Historical.—Anthrax,[1] or splenic fever (Fr., *charbon;* Ger., *Milzbrand*), is one of the best-known and longest studied of all bacterial diseases. As pointed out in the introductory chapter, the demonstration by Robert Koch[2] in 1876 of the causal relation between anthrax and a specific bacillus marks the beginning of modern bacteriology.[3] Prior to Koch's investigations, and as early as 1850, microscopic examinations by Davaine and Rayer,[4] Pollender,[5] and others had shown that a rod-like organism was constantly present in the blood and organs of animals dying from splenic fever, and inoculation experiments with the blood of infected animals, leading to a reproduction of the typical disease with all its symptoms and lesions, had been successfully performed by Brauell.[6] To the inferences drawn from these observations and experiments there was raised the objection that not the rod-like organisms, but something else in the diseased blood, might have caused the effects produced by blood inoculation. It must be conceded that this argument, although without experimental basis, was logically well founded. Before Koch's work, therefore, rod-shaped bacteria had been observed in the bodies of animals suffering from anthrax, and their etiologic connection with the disease had, in the judgment of many, been rendered highly probable; but it was not until Koch's researches appeared that medical opinion generally was impelled

[1] The disease of cattle known as "symptomatic anthrax" has nothing to do with true anthrax. (See p. 414.)

[2] Koch: Cohn's Beiträge, 1877, 2, p. 277.

[3] A fair example of the views upon the causation of anthrax prevailing at an earlier period is found in the hypothesis of Delafond, a French veterinary surgeon, who held that the anthrax of sheep was due to "an excess of blood circulating in the vessels." Concluding that this was caused by a rich nitrogenous pasturage, he advised sheep-raisers as a prophylactic measure to put the animals on short rations. (See Vallery-Radot: "Life of Pasteur," New York, 1926, p. 275.)

[4] Davaine and Rayer: Bull. Soc. de biol., 1850, p. 141.

[5] Pollender: Vierteljahr. f. ger. Med., 1855, 8, p. 103.

[6] Brauell: Archiv f. path. Anat., 1857, 11, p. 132.

to the conviction that the anthrax bacillus was the cause of the specific disease with which it was associated. Koch reached this result by obtaining the anthrax bacillus apart from all foreign matter and freed from any of the tissue fluids or body-cells of the diseased animal from which it was derived. This achievement was due to his discovery that the anthrax bacillus would grow and multiply outside the body upon the aqueous humor of the ox's eye. By cultivating it upon this medium for a series of generations, and, after allowing sufficient intervals for multiplication, transferring it from tube to tube, a growth was finally obtained which not only was not mixed with any of the blood-corpuscles or other matter derived from the original source, but was composed simply of the descendants of the original rod-like organisms many generations removed. Experiments made with this pure culture showed that a well-characterized attack of splenic fever, with the appropriate symptoms and lesions, could be produced by the introduction of a pure culture of bacilli into the body of a susceptible animal. Koch's observations upon the life-history of the anthrax bacilli also cleared up many of the difficulties and apparent paradoxes that had previously obscured the study of the disease. His discovery of the phenomenon of spore-formation, and of the part played by spores in the spread of the disease in nature, must be reckoned as one of the more important of these advances. It is known that previous workers had been greatly perplexed by the singular observation that occasionally specimens of blood which appeared not to contain any bacteria were nevertheless capable, on inoculation, of producing anthrax. This was satisfactorily explained by the discovery that in such cases spores, which are highly refractive and consequently visible with difficulty, had been formed in the blood after the blood had been drawn from the body. The prolonged vitality of the spores in soil, again, explained the persistence of the disease in certain localities and its reappearance in once-infected pastures after the lapse of many years.

Characteristics of the Anthrax Bacillus; Morphology.—The anthrax bacillus is one of the largest of the pathogenic bacteria and ranges from 4.5 μ to 10 μ in length and from 1 μ to 1.25 μ in breadth. In cover-slip preparations from the blood or lymph of an infected animal the rods are usually single, but rarely two or three are united in short chains Blood films and spleen pulp preparations when

stained by special methods (Johne,[1] Räbiger[2]) reveal the presence of a *capsule*. The bacilli stain readily with the ordinary anilin dyes and retain the stain when treated by Gram's method. The ends of the rods are often concave and somewhat swollen, so that the appearance of a chain of anthrax bacilli has been often compared to a jointed bamboo fishing-rod (Fig. 48). When grown on artificial culture-media, threads and filaments, sometimes of extraordinary length, are produced. After a varying period of growth, depending upon the temperature, nature of the nutrient medium, abundance of oxygen, and other factors, the highly refractive spores make their appearance in the interior of the rods.

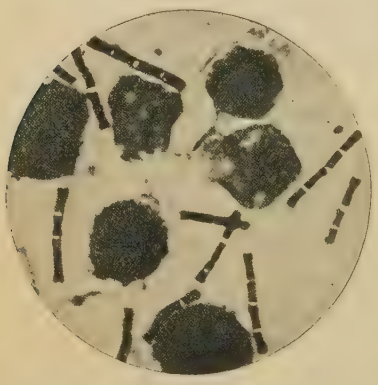


Fig. 48.—Bacillus anthracis in spleen pulp. Fuchsin stain; × 2000; C. Fränkel prep. (Kolle and Wassermann).

Spores and Spore-formation. Owing to the fact that the spores of the anthrax bacilli are among the most resistant forms of pathogenic bacteria, they have long been favorite test objects for determining the efficiency of germicides and other destructive agencies, and much attention has in consequence been directed to their morphologic and biologic properties. Only a single spore is produced in each cell. It is formed in the middle of the cell, where it can be seen in unstained preparations as an oval or nearly spherical, highly refractive body of the same diameter as the rod (Fig. 49). The chemical composition of the spores is said to differ from that of the rods in containing a larger proportion of fatty substances and protein (spores, 77.75 per cent. protein; rods, 42.5 per cent.). Spores are produced only in the presence of free oxygen, and hence do not occur in the circulating blood of infected animals, but develop either during the course of the infection, or after death, when blood is drawn and allowed to stand in contact with the air. Spores may be formed between 14° and 40° C., but are developed most abundantly at 32° to 35° C. Germination of the spore is usually

[1] Johne: Deut. Ztschr. f. Thiermed., 1893, 19, p. 244.

[2] Räbiger: Ztschr. f. Fleisch- u. Milchhyg., 1901, 11, p. 68.

polar, that is, parallel with the long axis, but may be rarely equatorial. Like all spores, those of the anthrax bacilli resist drying for a prolonged period (at least ten to twelve years). They are not so resistant to heat as the spores of the closely allied B. subtilis and some other saprophytic forms, and, as a rule, are killed by dry heat in three hours at 140° C., and by steam or boiling water in five to ten minutes, although some resist for much longer periods. Anthrax spores are able to withstand the action of the ordinary germicides much better than the bacilli; thus, a 10 per cent. solution of creolin kills anthrax bacilli in ten to twenty minutes, but is not able to effect the death of anthrax spores, the latter being able to maintain their vitality even in a 60 per cent. solution.

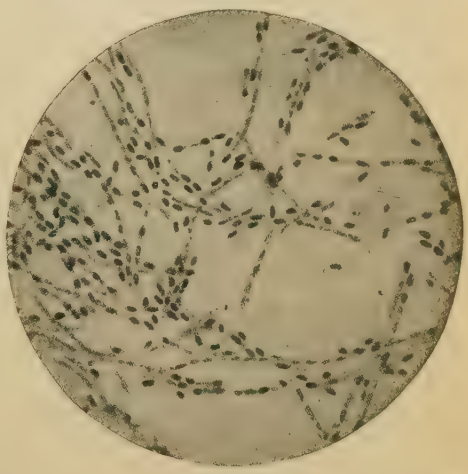


Fig. 49.—Bacillus anthracis with spores. Carbol-fuchsin and methylene-blue stain; × 1000 (Fränkel and Pfeiffer).

It is claimed that permanent asporogenous varieties of the anthrax bacillus have been obtained by various methods, such as growth in the presence of antiseptics (carbol-broth 1: 1000, Roux[1]), at high temperatures (42° C., Phisalix[2]), and under other disadvantageous conditions, and that such non-spore-forming races are in other respects entirely normal, even to exhibiting a full measure of virulence. Some of the statements in regard to this matter, however, lack entire corroboration, and it must at present be regarded as doubtful whether permanently asporogenous but fully virulent and undegenerate varieties of the anthrax bacilli either can be artificially produced or can exist in nature.

Growth Characteristics.—Upon the ordinary culture-media the anthrax bacillus grows freely under aërobic conditions and at ordinary temperatures. In broth, as a rule, no pellicle is produced on the surface, but a heavy flocculent sediment is formed, the inter-

[1] Roux: Ann. de l'Inst. Past., 1890, 4, p. 25.
[2] Phisalix: Archiv. d. Physiol., 1893, 5, p. 217.

vening layer of fluid in undisturbed cultures remaining quite clear; no indol is produced. The appearance of the colonies upon the surface of gelatin or agar plates is highly characteristic; long, wavy filaments project from the colony in every direction, and when viewed under the low power of the microscope, the thickly coiled masses have been likened to the snaky tresses of Medusa (Figs. 50 and 51). The same tendency to form filamentous outgrowths is seen in young gelatin stab-cultures, in which the "inverted fir-tree"

Fig. 50.—Deep (*a*) and surface (*b*) colony of anthrax on gelatin plate; × 80 (Flügge).

appearance is of common occurrence (Fig. 52). Gelatin is slowly liquefied. Milk is feebly acidified and is curdled by a rennet-like ferment, and the casein slowly peptonized. On potato a gray, furry growth is produced; spores are often formed in particular abundance on this medium.

Pathogenicity for the Lower Animals.—In nature anthrax is primarily a disease of cattle and sheep; horses and swine are susceptible, but are less commonly affected. In the German Empire in 1899 the following cases of anthrax were reported: 3678 cattle, 307 sheep, 282 horses, 61 swine, 6 goats. Wild deer and other gregarious herbivora are liable to occasional out-breaks. The smaller rodents are very sensitive to inoculation. Rabbits, guinea-

pigs, and white mice are susceptible in the order named, and are fatally affected by the subcutaneous introduction of a very small number of virulent bacilli. The white mouse may succumb to inoculation with a single germ of a virulent strain. Carnivorous animals, although possessing greater resistance than the herbivora, are nevertheless susceptible, as several epidemics in zoölogical gardens have shown, leopards, lions, pumas, bears, and others perishing from the disease (Jensen,[1] Lange[2]). Certain animals possess marked natural resistance to anthrax. Rats are quite resistant, especially the white rat, only about 14 per cent. of the latter dying as the result of inoculation. The dog is only slightly susceptible.

Fig. 52.—Bacillus anthracis' gelatin stab-culture (Hicks).

Fig. 51.—Bacillus anthracis, impression preparation, edge of colony; Zettnow prep. (Kolle and Wassermann).

Birds, especially pigeons, can be infected, but not easily. Frogs are immune, but toads very susceptible.

The route by which the germs enter the body exerts an important influence in both experimental and natural infection. Subcutaneous inoculation is the method most commonly practised in experimental work, and is almost uniformly fatal with the ordinary small laboratory animals. It has been frequently stated that intravenous and intraperitoneal inoculations are even more constantly and surely

[1] Jensen: Baumgarten, Jahresb., 1891, 7, p. 167 (cited).
[2] Lange: Hyg. Rundsch., 1901, 11, p. 529.

fatal than subcutaneous, but late researches cast doubt upon this and indicate that if great pains be taken to prevent subcutaneous infection during the course of the operation, animals can withstand the introduction of a considerable number of anthrax bacilli directly into the circulation or into the peritoneum. Feeding experiments show that administration of spore-free cultures even to highly susceptible animals is without result, owing to the destruction of the bacilli in the stomach. The feeding of spores, on the contrary, leads to infection of the more susceptible species, although not so certainly as subcutaneous inoculation. The more resistant species (for example, swine) are with difficulty infected through the alimentary tract. Infection through the respiratory tract is apparently possible, so far as the experimental results indicate, although it is probably almost unknown in the lower animals under ordinary conditions, and the views of experimenters are not wholly in accord.

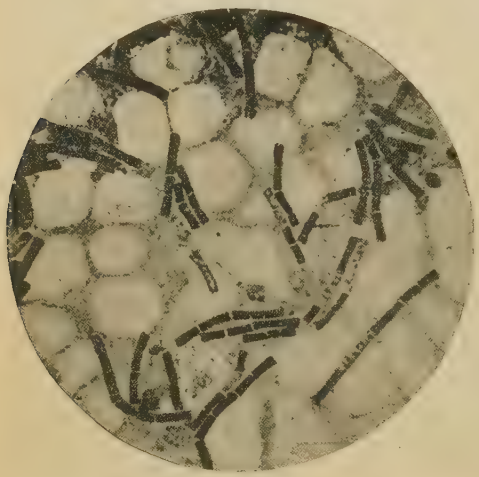


Fig. 53.—Bacillus anthracis in peritoneal fluid of a mouse; gentian-violet; × 1000 (Fränkel and Pfeiffer).

In very susceptible animals the disease runs a rapid course and presents all the characteristics of a typical septicemia (Fig. 53). Local manifestations may be almost entirely absent. Enormous multiplication of the bacteria takes place in the blood and internal organs, and sections through the liver or spleen show that the capillaries are gorged with masses of bacteria (Fig. 54). The spleen is of a deep-red color and is greatly enlarged, whence the appropriateness of the name splenic fever as applied to this disease in cattle. The more resistant animal species do not develop this generalized infection, but the bacteria remain localized in an abscess or carbuncle and fail to spread extensively through the body. This is the case in the dog and, as will be seen presently, in man also with certain forms of infection. In this respect anthrax furnishes an

illustration of the general rule that when a bacterial invasion meets slight resistance from the animal tissues an abundant multiplication of the bacteria occurs throughout the body, while the possession of high powers of resistance is accompanied by a pronounced local reaction. Man stands perhaps midway in susceptibility between the dog and the sheep.

Under natural conditions cattle and sheep are infected through the alimentary tract by swallowing spores while grazing in infected pastures. As has been pointed out, spores are able to retain their vitality in soil for a long period, and pastures once infected with the disease are able to infect cattle after the lapse of many years (thirty years). Hides imported from China and other countries where the disease prevails are not uncommonly contaminated with anthrax spores; in the United States several outbreaks of anthrax among cattle with some consequent cases of human infection have been traced to the overflowing of pasture land by streams receiving the drainage of tanneries.[1]

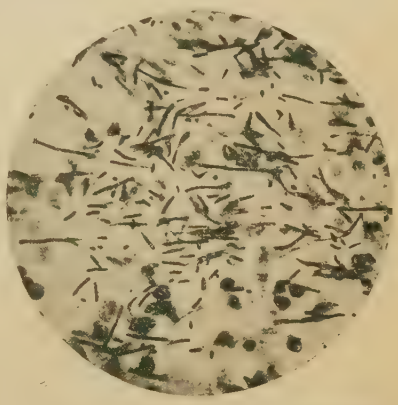


Fig. 54. — Bacillus anthracis. Section of spleen of mouse; × 500 (Günther).

Cattle may also occasionally be infected by direct contact through wounds, abrasions, and other injuries to the skin; but alimentary tract infection is by far the more usual. Anthrax has been experimentally conveyed to susceptible animals by biting flies of various species that had previously fed on animals dying from anthrax.[2]

Pathogenicity for Man.—Three modes of infection of human beings are known to exist—(*a*) through the skin, (*b*) through the respiratory tract, and (*c*) through the alimentary tract. The bacillus is almost always transmitted to man through the agency of the lower animals rather than through other human beings. The

[1] Ravenel: Rept. Amer. Pub. Health Assoc., 1898, 24, p. 302; Russell, Seventeenth Ann. Rept. of Wisconsin Agr. Expt. Station, 1900, p. 171; Gärtner and Dammann, Arb. a. d. k. Gesund., 1907, 25, p. 416.

[2] Mitzmain: Bull. 94, Hyg. Lab., U. S. Pub. Health Service, June, 1914.

persons most commonly affected are those having to do with cattle and their products, such as butchers, shepherds and herdsmen, handlers of hides, hair, and fleeces. Laboratory infections, sometimes fatal, have been known to occur with pure cultures of the anthrax bacillus.

In the United States 222 human deaths from anthrax were recorded in the eight years, 1910–17. The majority of these occurred in the three years, 1915–17, when the interference with the usual channels of import caused by the Great War, combined with scarcity of labor, led to a less efficient preliminary disinfection, and so permitted the introduction of anthrax-contaminated hides and bristles from parts of Asia and South America. A particularly striking increase in anthrax occurred from the use of shaving brushes, and in a number of instances anthrax bacilli were found in brushes purchased on the open market. The United States Public Health Service recommends soaking new brushes for four hours in a 10 per cent. solution of formalin. The solution should be kept at a temperature of 110° F. and the brush so agitated as to bring the solution into contact with all hair or bristles.

The case fatality of anthrax is not exactly known, but is probably not far from 20 per cent.

(*a*) *Malignant Pustule.*—The most common form of anthrax in the human subject is due to skin infection, and usually takes the form of localized boil or abscess, which often heals spontaneously, but may progress into a septicemic condition unless checked by incision or other surgical procedure. The handling of infected hides or carcasses constitutes the ordinary means of infection. Porters on the London docks, who carry on their naked backs hides imported from South American or Asiatic ports, sometimes develop malignant carbuncles as a result of anthrax infection through dorsal abrasions or scratches. Twenty-five cases of anthrax, due to handling hides imported from China, occurred in Massachusetts between March and June, 1916.[1] Owing to the relatively high resistance of man, septicemia does not often occur, especially if the carbuncle be incised and thoroughly cleansed. Lesions of all sizes many be produced, from a minute pustule to a large and angry abscess.

(*b*) *Pulmonary Anthrax.*—The pulmonary form of anthrax due to inhalation is the most dangerous, although not the most com-

[1] Brown and Simpson: Jour. Amer. Med. Assoc., 1917, 68, p. 608.

mon, variety of the disease in man. The name currently applied to this variety of anthrax in England is "woolsorters' disease," and, as the name implies, the affection is usually caused by inhalation of the spores set floating in the air during the handling and sorting of wools and fleeces. It is characterized by many of the symptoms of pneumonia and often passes into a fatal septicemia.

(*c*) *Intestinal Anthrax.*—The alimentary tract, although the usual path of infection in cattle, is very rarely so in man. A few instances are on record of the causation of intestinal anthrax through the medium of spore-infected food. Such cases have usually occurred among workers with animal products, and have probably been due to lack of caution in handling food with uncleansed hands. Insufficiently cooked meat from anthrax-infected animals may also be a source of intestinal anthrax.

Mode by which the Anthrax Bacillus Causes Injury to the Animal Organism.—In the typical form of anthrax septicemia, bacilli are found in immense numbers clogging the capillaries, and apparently by their accumulation hindering the circulation of the blood. This fact caused the theory to be advanced very early that death in such cases was due to a kind of internal suffocation. This view, however, finds no support in the character of the symptoms of anthrax. Death, moreover, frequently occurs in some animals in the absence of any noteworthy number of bacteria within the blood-vessels. This is particularly true in fatal cases of anthrax in man. It might seem from analogy that we should find that the anthrax bacillus secretes a soluble toxin such as is formed by the diphtheria bacillus and some other pathogenic microbes, and many of the phenomena of anthrax infection seem to point to the action of such a substance. All attempts, however, to demonstrate the existence of either an extracellular or an intracellular anthrax toxin have been unsuccessful, and, although all the probabilities are in favor of the existence of some such substance, the exact manner in which the anthrax bacillus damages the animal organism remains at present a mystery.

Immunity.—Some natural susceptibility to anthrax is possessed by many animals; the degree of such susceptibility may be heightened or diminished by a great variety of factors. Normal susceptibility, for example, may be lessened by the injection of thymus extract and other organic substances, and also by certain operative

procedures, such as section of the sciatic nerve. Susceptibility may be increased by a number of physiologic influences, such as alteration of the normal body-temperature, as in Pasteur's classic experiment with refrigerated fowls, which under normal conditions are immune to inoculation, but succumb when chilled; the frog, conversely, which is normally without susceptibility, becomes susceptible when kept at a high temperature. Lowering the temperature of mammals with drugs, such as antipyrin, has a depressing influence on the power of resistance. Administration of other drugs, such as alcohol, phloridzin, and chloroform, feeding with unsuitable or insufficient food, subjection to excessive fatigue, and other factors all increase susceptibility.

The cause of the high natural immunity to anthrax possessed by the dog, fowl, and certain other animals has been the object of much experimentation. No antitoxin has been demonstrated in the blood of naturally immune animals. The body-fluids of some species manifest bactericidal powers toward the anthrax bacillus, but there is no concurrence between the degree of immunity and the anthracidal power of the blood-serum. The blood-serum of the highly susceptible rabbit is strongly bactericidal outside the body, but anthrax bacilli injected into the circulation seem to multiply freely in the blood-stream. Blood taken from the very resistant dog and fowl is practically devoid of bactericidal properties. Bail and Petterson[1] have attempted to explain the absence of bactericidal power in the drawn blood of the latter animals by supposing that the constituent known as the complement, which, within the dog, is supplied by the leukocytes, is lacking in the drawn blood. As evidencing this, they show that addition of a suitable complement to dog's serum, either in the guise of rabbit serum or of canine leukocytes, imparts to the dog's serum a strongly germicidal power. On the other hand, the bactericidal action manifested by rabbit serum outside the body is supposed to be restrained within the body by the presence of a substance which binds the amboceptor and so prevents the destruction of the anthrax bacilli. Future investigation must determine how far this view is correct.

The share of phagocytosis in natural immunity is likewise under discussion. As has been shown by Hektoen,[2] it is highly probable

[1] Bail and Petterson: Centralbl. f. Bakt., 1903, I, Orig., 33, p. 756.

[2] Hektoen: Jour. Amer. Med. Assoc., 1906, 46, p. 1407.

that the natural immunity of the dog is due to phagocytosis. The destruction of virulent anthrax bacilli that takes place in mixtures of normal serum and washed blood-corpuscles is accompanied by marked phagocytosis. Neither normal serum alone nor suspensions of washed corpuscles can prevent the multiplication of the bacteria, and it seems reasonable to conclude that the normal serum contains an opsonin or sensitizing substance which prepares the bacilli for the onslaught of the phagocytes. Other experimental evidence for this view is contained in the article just cited.

Vaccination Against Anthrax.—Animals naturally susceptible may be made immune by artificial means, and domestic animals have been largely protected against anthrax in this way. Pasteur devised a method for vaccinating cattle and sheep against anthrax which is dependent on the subcutaneous inoculation of attenuated cultures. Two vaccines were used. The "first vaccine" consisted of a broth culture whose virulence was so greatly diminished by heat that it would no longer surely kill guinea-pigs, although it was still fatal for white mice. After twelve days a second inoculation was made with the "second vaccine," which was of such a strength that it would kill guinea-pigs but not rabbits. Following inoculation with these two vaccines, a fully virulent culture could be injected with impunity. In spite of some accidents due to the use of imperfectly standardized vaccines, this method of protective inoculation has proved, on the whole, of great practical value. In France 30,000 to 50,000 cattle and horses and 250,000 to 350,000 sheep are vaccinated annually. It is estimated that many thousands of animals are saved by this procedure. Active immunization of rabbits and guinea-pigs can also be effected by the injection of attenuated cultures, but with much greater difficulty.

The simultaneous inoculation of anti-anthrax serum and a spore vaccine has been quite extensively practised in the United States in districts where anthrax is prevalent. The method is not without risk.

The serum of actively immunized animals contains specific protective substances. Inoculation with the blood-serum of an actively immunized animal confers some degree of protection against anthrax infection, and in the hands of Sobernheim,[1] Sclavo,[2]

[1] Sobernheim: Ztschr. f. Hyg., 1897, 25, p. 301; Centralbl. f. Bakt., I, 1899, 25, p. 840.

[2] Sclavo: Centralbl. f. Bakt., 1899, 26, p. 425.

and others has been attended as well with some measure of therapeutic success; that is, injection of the serum will save the life of animals even when the anthrax bacilli have already entered the circulation. Very favorable reports of the use of Sclavo's serum in cases of anthrax in man have come from Italy and from South America. Eichhorn's serum[1] has been used with success in a number of human cases in the United States.

The mechanism by which the protective serum exerts its action is not certainly known. With our present knowledge, perhaps the most reasonable view is to regard anthrax immunity as a phagocytic immunity and the function of the immune serum as sensitizing or opsonic.

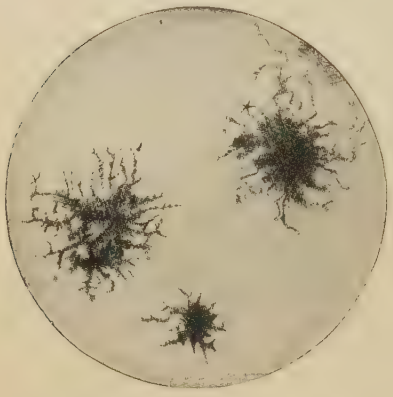


Fig. 55.—Bacillus subtilis from panophthalmitis; agar colonies; × 30 (Axenfeld).

Bacillus Subtilis.—The common bacillus of hay infusion, B. subtilis, is culturally and morphologically very similar to B. anthracis. The spores, however, germinate equatorially instead of at the pole. A heavy tenacious pellicle is formed in broth cultures; gelatin and casein are liquefied more rapidly than by B. anthracis. B. subtilis is widely distributed in earth, air, and water, and was formerly regarded as one of the most typical of "non-pathogenic" organisms. The first report of any pathogenic power on the part of this germ was made by Charrin and de Nittis,[2] who cultivated B. subtilis upon blood-media and passed it through animals until it had acquired considerable virulence. The hay bacillus is also capable of producing spontaneous infection in man, as shown by Baenziger and Silberschmidt,[3] who found the organism in pure culture in a case of panophthalmitis following penetration of the cornea by a piece of steel. They reproduced the disease in rabbits and regained the organism. More recently Silberschmidt[4]

[1] Jour. Agr. Res., 1917, 8, p. 37.

[2] Charrin and de Nittis: Compt. rend. Soc. de biol., 1897, 49, p. 711.

[3] Baenziger and Silberschmidt: "Bericht der ophthal. Gesellschaft," Heidelberg, 1902.

[4] Silberschmidt: Ann. de l'Inst. Past., 1903, 17, p. 268.

and others[1] have described several such cases (Fig. 55). The toxic result of infection is believed to be due to some substance in the bodies of the bacilli, since the filtrates of cultures have little or no effect.

Before methods of studying bacteria had become as elaborate and well tested as they now are, the close resemblance of B. subtilis and B. anthracis led to a belief that these organisms were simply closely related varieties and could be transformed one into the other. Buchner in 1880 maintained that he had succeeded in changing the virulent anthrax bacillus into the common hay bacillus and *vice versâ*. His assertions seem to have been based largely upon the fact that the anthrax bacillus may be attenuated or made less virulent by a variety of methods, and also upon an insufficient knowledge of the biologic differences between the two species.

The so-called potato bacillus (B. mesentericus) and a number of other aërobic spore-bearing bacilli found in water and soil may be ranked in the B. subtilis group.

[1] Kayser: Centralbl. f. Bakt., 1903, I, Orig., 33, p. 241; Kneass and Sailer: Univ. Penn. Med. Bull., 1902, 16, p. 131.

CHAPTER XV

THE DIPHTHERIA BACILLUS[1]

Genus: Corynebacterium. Parasites. Slender, often slightly curved, rods with tendency to club formation. Barred irregular staining. Not acid-fast. Gram-positive. Non-motile. Aërobic. No endospores. Powerful exotoxin.

The discovery of the bacillus of diphtheria and the study of this organism and its products have profoundly affected both the mode of treatment of the disease and the manner of combating its spread.

(1) Early recognition of the real nature of a throat infection is necessary for proper treatment, and we are now able to distinguish with certainty the highly dangerous class of throat affections caused by the diphtheria bacillus from clinically similar but less dangerous anginas that are due to a different cause. In a word, the finding of B. diphtheriæ enables a correct diagnosis to be made. (2) Investigation of the physiologic properties of the diphtheria bacillus has led directly to the discovery of diphtheria antitoxin, a specific remedy of unquestioned value. (3) By systematic bacteriologic examination of the throats of convalescents it is possible for health officials to fix the term of necessary quarantine with much more precision than formerly. The power to do this is an invaluable aid in limiting the spread of the disease. (4) Inoculation of a minute amount of toxin into the skin (Schick test, p. 302) makes it possible to distinguish susceptible children from resistant; the former can then be protected against diphtheria by a toxin-antitoxin mixture.

The earliest description of the diphtheria bacillus appears to have been given by Klebs[2] in 1883, but the etiologic relations of this organism first came into notice through the investigations of

[1] The generic name Corynebacterium was bestowed on the diphtheria bacillus by Lehmann and Neumann in 1896, and was adopted in the classification of the Committee of the Society of American Bacteriologists. Sixteen species including the acne bacillus, the bacillus of pseudotuberculosis in guinea-pigs, the xerosis bacillus and other diphtheroids are included in this genus in the 1925 edition of Bergey's Manual.

[2] Klebs: Verhandlungen des Congresses f. innere Med., 1883.

Löffler, published in 1884.[1] Löffler succeeded in obtaining in pure culture from a number of cases of diphtheria the bacillus seen by Klebs. Although Löffler's observations favored the view that the bacillus thus cultivated was the cause of diphtheria, Löffler expressly disclaimed the assumption that this was actually the case, largely on the ground that the bacillus was not found in all cases of clinical diphtheria, while, on the other hand, it had been found by him in the throat of a perfectly healthy child. The significance of such findings is now more clearly understood. A similarity of clinical symptoms does not always betoken causal identity. So far as the local manifestations are concerned, streptococci can produce a condition apparently indistinguishable from that in which the Klebs-Löffler bacillus is found. Again, it is now known that the diphtheria bacillus is occasionally present in the healthy throats of persons associated with diphtheria patients. Continued investigation by various observers showed that the Klebs-Löffler bacillus was always present in the typical false membrane of diphtheria, and in 1888–89 Roux and Yersin[2] triumphantly demonstrated the etiologic relation of the bacillus to the disease by showing that it formed a toxin which was capable of reproducing with singular fidelity the characteristic symptoms and lesions.

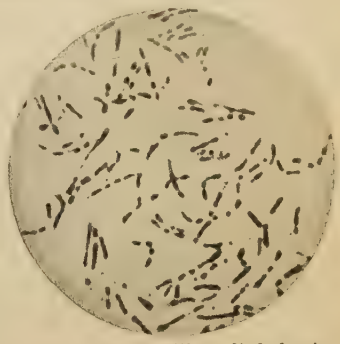


Fig. 56.—Bacillus diphtheriæ, fifteen-hours serum culture. Löffler's methylene-blue; × 2000 (Denny, in Journal of Medical Research).

Morphology.—The diphtheria bacillus is a slender rod ranging from 1 μ to about 6 μ in length. When stained with Löffler's methylene-blue, it usually presents a beaded, striated, or granular appearance, which is so characteristic that by simple microscopic examination a trained observer can recognize the Klebs-Löffler bacillus in cultures from a suspicious throat (Fig. 56). In cover-slip preparations made directly from the false membrane the uneven staining of the cell protoplasm is less noticeable than in preparations from cultures. Club-shaped forms are sometimes observed in films made from the membrane, but less frequently than in films from

[1] Löffler: Mitt. a. d. k. Gesund., 1884, 2, p. 421.

[2] Roux and Yersin: Ann. de l'Inst. Past., 1888, 2, p. 629.

nutrient media; in the latter, bacilli with swollen and deeply stained ends are, as a rule, abundant (Fig. 57). Bacilli containing metachromatic granules are commonly observed in cultures derived directly from clinical cases. Many recent observers recognize

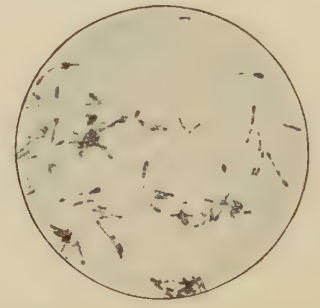


Fig. 57.—Bacillus diphtheriæ; blood-serum culture showing clubbed ends and irregular staining; methylene-blue; × 1100 (Park).

different morphologic types of B. diphtheriæ. In the nose and throat of both healthy and diphtheritic persons diphtheria bacilli, known as the "barred" and "solid" types, are found (Wesbrook[1])

Fig. 58.—Wesbrook's types of Bacillus diphtheriæ: *a*, *c*, *d*, Granular types; a^1, c^1, d^1, barred types; a^2, c^2, d^2, solid types; × 1500.

(Fig. 58). The distinction is based upon a difference in behavior toward stains. The protoplasm of the so-called solid form stains uniformly and shows neither bars nor granules. The protoplasm

[1] Wesbrook, Wilson, and McDaniel: Trans. Assoc. Amer. Physicians, 1900; see also Gorham: Jour. Med. Res., 1901, 6, p. 201.

of the barred form stains in irregular blocks or segments, the intervening portion taking the stain slightly or not at all. The barred or striated type is said to be frequently present on the conjunctiva, and when isolated from a form of conjunctivitis known as xerosis, is usually denoted as the *xerosis bacillus*. The term xerosis bacillus has, however, been loosely used, and a number of different kinds of organisms have been sometimes put under this head. A diphtheroid organism which has been named B. segmentosus, and is perhaps the cause of certain acute cases of rhinitis, also belongs in this group.[1] It is stated that barred forms are sometimes found in clinical diphtheria as the sole type, but this condition appears to be very rare. The relation of the solid type to clinical diphtheria is still obscure. By some authorities the solid forms are classed as pseudodiphtheria bacilli and are not regarded as capable of causing diphtheria. The barred and solid types are found much more commonly than the granular type in the nose and throat of healthy individuals.

The granular type, on the other hand, predominates in clinically characteristic diphtheria. Several observers report that during convalescence the granular type is gradually replaced by the solid, a fact that has been regarded as pointing to a gradual morphologic alteration brought about by the influence of the body-fluids of an immune individual.[2]

In some cultures of the diphtheria bacillus true branching has been observed. The branching apparently originates by budding, and is sometimes followed by degeneration of the parent stem at the point of origin. Sometimes very complex branching forms are produced (Hill). By the use of the hanging-block method of cultivation Hill[3] observed so-called post-fission movements in B. diphtheriæ. After cell division a sudden snapping across of the rod occurs, which seems to be strictly characteristic of the organisms of this group.

The diphtheria bacillus exhibits a marked tendency to the production of involution forms. These occur with special abundance in cultures on artificial media: for example, on blood-serum after

[1] Walter: Jour. Amer. Med. Assoc., 1910, 55, p. 1091.

[2] An excellent discussion of morphologic types of B. diphtheriæ and their significance in public health work is given in the Amer. Jour. of Pub. Hyg., 1907, 17, p. 156.

[3] Hill: Jour. Med. Res., 1902, 7, p. 202.

five to seven days. The biologic significance of the appearance of involution forms as well as of branching forms among the diphtheria bacilli and certain other groups of bacteria is not at present understood.

B. diphtheriæ does not form spores and does not possess the power of movement. When tested by Gram's method most races retain the stain.

Gins[1] has modified Neisser's granule stain for diphtheria bacilli, using Lugol's solution after Neisser's solution No. 1 (p. 56). Scheller[2] recommends lengthening the action of each stain by from ten to fifteen seconds. When this staining method is applied to cultures grown upon Löffler's serum the method gives fairly good results, and undoubtedly aids in diagnosis; but it does not permit the sharp distinction to be made between diphtheria and pseudo-diphtheria bacilli that was originally claimed for it. Many other special methods of staining this organism have been employed with more or less success, but few of them are so satisfactory as the long-used Löffler methylene-blue (see p. 50).

Cultural Characteristics.—The diphtheria bacillus grows fairly rapidly upon appropriate nutrient media, provided a suitable temperature—not less than 19° C.—be maintained. The optimum is about 37° C. The reaction of the medium is a feature to which this organism is peculiarly sensitive, a reaction that is about P_H7. 8-8. 2 being most suitable both for growth and for toxin production. An abundant oxygen supply is likewise a requisite. The blood-serum medium recommended by Löffler is ordinarily employed for isolation. *Löffler's serum* consists of a mixture of three parts of calf or sheep serum with one part of 1 per cent. glucose broth. On this medium the diphtheria bacilli grow rapidly at 37° C., often forming minute but visible colonies inside of twelve hours; within eighteen to twenty hours small opaque gray colonies are plainly seen on the surface, and from a microscopic examination of cover-slip films made from these colonies diagnosis is almost invariably possible. Other organisms that may have been present in the throat or in the false membrane are usually outstripped by the diphtheria bacillus in growth on Löffler's serum. Upon ordinary nutrient gelatin and agar the diphtheria bacillus is also able to develop, but less luxuriantly; gelatin is not liquefied by the growth.

[1] Gins: Deut. med. Wchnschr., 1913, 39, p. 502.

[2] Scheller: Kolle and Wassermann, Handbuch, Ergänzungsband 2, p. 107.

The surface colonies on agar, when viewed with a low magnification, are coarsely granular and somewhat irregular in outline, with ragged or fringed edges (Figs. 59 and 60). In milk abundant growth occurs with feeble acid reaction, but the lactose is not fermented and no curdling of the milk takes place. The acid reaction of potato is not favorable to growth. In broth containing dextrose an acid reaction is produced by the majority of the granular, virulent forms, while nearly all of the solid-staining forms that are encountered

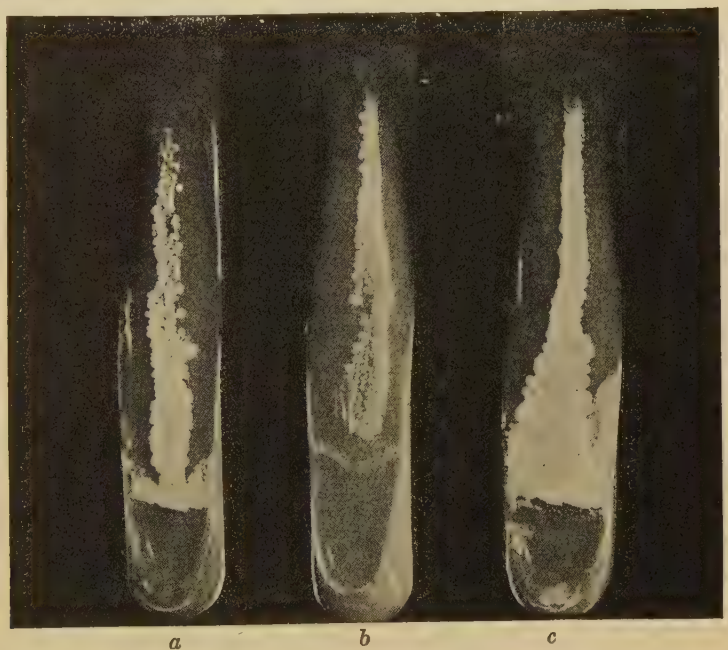


Fig. 59.—Diphtheria bacilli (from photographs taken by Prof. E. K. Dunham, Carnegie Laboratory, New York): *a*, Pseudobacillus; *b*, true bacillus; *c*, pseudobacillus.

are unable to ferment dextrose. The distinction, however, does not seem to be an absolute one. Nitrate is reduced to nitrite by most strains of B. diphtheriæ; indol is not produced.

Resistance.—In growths upon the ordinary culture-media the bacillus may retain its vitality for a long time. On agar it may live for six to eight weeks, on ordinary blood-serum, five to six months, and on dextrose blood-serum (Beck[1]), twelve to fifteen months. Although virulence ordinarily becomes lessened in cultures, many strains conserve their full virulence under prolonged

[1] Beck: Kolle and Wassermann, Handbuch, 2, p. 777.

cultivation. Löffler has recorded one instance where virulence was maintained during seventy-seven transfers, covering a period of twenty-seven months. In the dried diphtheritic membrane life can be maintained for a long period. Some observers have isolated bacilli from fragments of dried membrane after the lapse of several months.

Heat kills the bacilli rather readily. According to Brieger and Fränkel,[1] exposure to moist heat for forty-five minutes at 55° C. proves fatal. In the dry membrane they are much more resistant and are said to have withstood exposure to 98° C. for an hour.

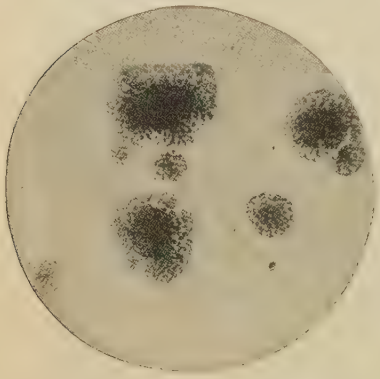


Fig. 60.—Bacillus diphtheriæ colonies on glycerol-agar, twenty-two hours old; × 39 (Heim).

The Diphtheria Bacillus in the Human Body.—Mucous surfaces are a favorite site for the growth of the diphtheria bacillus. Swabbing the throats of adult humans volunteers with a pure culture of the diphtheria bacillus has been followed promptly by cases of clinical diphtheria.[2] The pharynx is the locality most commonly affected, but diphtheria of the larynx or membranous croup, and nasal diphtheria or membranous rhinitis, are by no means infrequent. The nose probably often serves as the portal of entry. Diphtheria of the conjunctiva sometimes occurs as the result of a diphtheritic patient coughing or sneezing into the eyes of the attendant physician or nurse. Diphtheritic infection of the middle ear is not uncommon. Infection of the mucous surfaces of the genital organs is occasionally met with. Primary infection of the lungs may occasionally occur, but is very rare. Apparently simple "colds" are sometimes due to the diphtheria bacillus. Although the diphtheria bacillus sometimes finds its way into the general circulation and gives rise to septicemia, it remains, as a rule, confined to the mucous surfaces. The symptoms and lesions produced are due partly to the presence of the bacillus and partly to its toxin.

[1] Brieger and Fränkel: Berl. klin. Wchnschr., 1890, 27, p. 241.

[2] Guthrie, Marshall and Moss: Bull. Johns Hopkins Hosp., 1921, 32, p. 369.

The chief local consequence of infection is a degeneration of the epithelial cells, extending to the underlying tissues and accompanied by a profuse fibrinous exudation. As a result the characteristic diphtheritic membrane, containing fibrin, dead tissue-cells, leukocytes, and bacteria, is formed on the affected surface. The diphtheria toxin doubtless has a share in the formation of the membrane, but the most serious injuries that it causes are the systemic lesions due to its absorption. Diphtheria is a typical toxemia. The most severe lesions are produced in the heart, nerves, and kidneys. A variety of lesions may be found in the kidneys, acute interstitial nephritis being the most common. It is an old observation that albumin is found quite often in the urine of diphtheria patients; there is therefore no reason for attributing this symptom to the administration of diphtheria antitoxin; as a matter of fact, tests have shown that the amount of albumin in the urine is sometimes diminished after antitoxin is given. The lesions in the heart consist commonly of a fatty degeneration of the muscle-fibers, which may be very extensive. Fatty degeneration also occurs both in the myelin sheath of the peripheral nerves and in the white matter of the brain and cord. These changes in muscle and nerve seem to explain the nature of the grave cardiac weakness often observed in diphtheria, and also the frequent occurrence of the more or less extensive paralysis which so commonly follows a diphtheritic attack. Here too lies the explanation of the sudden fatal termination of many cases of diphtheria regarded as "mild" or even not recognized as true diphtheria. A small amount of toxin can probably cause extensive damage to vital tissues. "A patch of membrane the size of a thumb-nail on the tonsil may generate sufficient toxin to cause death" (McCollom[1]).

Animal Inoculations.—Both the general and local symptoms of diphtheria can be reproduced by animal inoculation. Guinea-pigs are readily killed by subcutaneous injection of a young broth culture, death usually occurring within two or three days after they are inoculated with a few drops of a twenty-four-hour broth culture of a virulent strain. Nephritic symptoms, paralytic manifestations, and other characteristic features of human diphtheria have been observed in the guinea-pig and other animals. An enlarged and hemorrhagic condition of the adrenals characterizes

[1] McCollom: Osler's "Modern Medicine," Phila., 1907, 2, p. 411.

diphtheritic intoxication in guinea-pigs. Paralytic manifestations appear more frequently in dogs and in pigeons than in guinea-pigs or rabbits. As a rule, the bacilli remain localized and are not found in large numbers in the internal organs of the infected animals. Inoculations upon the healthy mucous membrane of most adult animals lead to no changes, but if young animals be injected intratracheally or if the mucous surface be injured before inoculation a characteristic false membrane is produced. The membrane produced experimentally is histologically identical with that found in cases of human diphtheria. Animals vary considerably in their susceptibility to infection. Rats and mice are relatively refractory; rabbits are less susceptible than guinea-pigs; cats, dogs, and pigeons are highly susceptible. Bacilli introduced into the alimentary tract have no pathogenic effect, owing doubtless to their inability to effect a lodgment on uninjured epithelial surfaces. The bacilli are never found in any considerable abundance in the blood, and they appear only exceptionally to invade the tissues; injury to the organism, as a rule, results from the absorption of the toxin formed in the false membrane rather than from the presence of the bacilli in important organs.

The animal test for virulence, which must always be made for the final identification of an organism suspected of being the diphtheria bacillus, is commonly made by injecting 1 c.c. of a forty-eight-hour broth culture into each of two guinea-pigs. One of the animals is injected at the same time with 50 to 100 units of diphtheria antitoxin. If the bacillus is a true toxin-producing diphtheria bacillus the unprotected animal will die within two to three days, and typically congested adrenals will be found at autopsy; the protected guinea-pig will live.

The use of the original diagnostic culture (whole-culture method) injected intracutaneously saves time and appears to be fully as accurate.[1] The growth of the diagnostic culture is suspended in 1 c.c. salt solution and diluted further according to the number of diphtheria-like organisms present, the final volume of the suspension being from 1 to 4 c.c. One-tenth of a cubic centimeter of the suspension is injected into the skin of the abdominal region of a guinea-pig. The animal is observed for three days or until charac-

[1] Haven and Powell: Amer. Jour. Hyg., 1922, 2, p. 237; Bull and McKee: Amer. Jour. Hyg., 1923, 3, p. 103.

teristic lesions—necrosis of the superficial layer of the skin—develop. Four to six cultures may be tested on one animal.

The Diphtheria Toxin.—When B. diphtheriæ is grown in ordinary nutrient broth a soluble toxin is formed which diffuses out from the bodies of the living bacilli into the surrounding medium. The toxin is present in hardly diminished strength in the sterile filtrate of a broth culture. A persistent acid reaction interferes with the formation of toxin, and hence, if sugar is present in the broth in considerable quantity, the acid produced by its fermentation hinders materially the accumulation of toxin. In order to obtain maximum toxin production, the earlier experimental procedures involved passing a current of air over the surface of broth cultures, the purpose of this being to facilitate the oxidation of the acid products of growth. The use of broth freed from muscle-sugar (p. 32) renders this device less essential, although the free access of air to the cultures is still recognized as important. The broth for toxin production should be a thin layer in a wide-mouthed flask. Theobald Smith[1] has shown that the addition of a very small amount of dextrose, not exceeding 0.2 per cent., to broth previously freed from muscle-sugar favors toxin production. Other conditions, such as a temperature of about 36° C. and an abundance of peptone (2 per cent. Witte's peptone), or other albuminous substances and a well-developed surface growth, favorably influence toxin production. The toxicity, as a rule, attains a maximum in about five to ten days, depending upon the race used. Different races of diphtheria bacilli vary strikingly in their toxin-forming power. Certain strains that have been found to yield permanently an especially potent toxin are in widespread use in establishments for the manufacture of diphtheria antitoxin. Some of these strains generate so powerful a toxin that 0.001 c.c. of a filtered broth culture proves fatal to a guinea-pig, while other strains may generate so little that several cubic centimeters are necessary to produce a fatal result.

Little or nothing is known regarding the chemical nature of diphtheria toxin. It is destroyed by boiling for five minutes, and is greatly weakened by lower temperatures (60° to 70° C.). Direct sunlight causes a complete loss of toxicity within a few hours. When the toxin is kept in the dark and in cold storage, it may retain

[1] Smith, Theobald: Jour. Exper. Med., 1899, 4, p. 373.

its activity for a long period—according to Abba[1] as long as two years. Roux and Yersin[2] showed that the addition of a small amount of acid to a toxic filtrate caused the toxic property to disappear, but that if the acid were not allowed to act too long, the toxicity was regained on restoring the original reaction. The toxic body is precipitated by alcohol and by calcium chlorid, calcium phosphate, ammonium sulfate, and other protein precipitants, but it is probably not itself a protein substance. Neither is the presence of protein necessary for its formation. Guinochet[3] has shown that toxin is formed when diphtheria bacilli are grown in urine that is free from albuminous constituents. Uschinsky found that toxin is produced in a protein-free medium containing only ammonium lactate, sodium asparaginate, glycerol, and some simple mineral salts.[4]

The toxin in some respects resembles an enzyme, but objections have been urged against the view that it is itself an enzyme. Such are its relatively high resistance to heat, the direct relation between the amount of toxin and the toxic effect produced as compared with the unlimited capabilities of true enzymes (Behring[5]), and other features not in harmony with the ordinary conceptions of enzymes.

The effects produced in animals by inoculation with a sterile diphtheria toxin are wonderfully similar to those produced by infection with living bacilli, save that no false membrane is formed by the toxin alone. The other symptoms that are evoked are practically identical whether bacilli or toxin be employed. The histologic lesions which occur in the heart and other organs, and are found both in cases of human diphtheria and in animals inoculated with diphtheria bacilli, are reproduced by the germ-free toxin. There is no escape, therefore, from the conclusion that in diphtheria the chief injury to the animal organism is brought about by the action of a potent poison which is secreted during the life of the bacillus and diffuses from the bacterial cell into the surrounding medium.

[1] Abba: Centralbl. f. Bakt., 1898, 23, p. 934.

[2] Roux and Yersin: Ann. de l'Inst. Past., 1889, 3, p. 273.

[3] Guinochet: Archiv. de med. exp., 1892, 4, p. 487.

[4] Hadley (Jour. Infect. Dis., Supplm. No. 3, 1907, p. 95) obtained abundant toxin production in sixteen days on the following medium: sodium chlorid, 0.6 per cent.; calcium chlorid, 0.08 per cent.; magnesium sulfate, 0.32 per cent.; dipotassium phosphate, 0.23 per cent.; ammonium acetate, 0.75 per cent.; glycerol, 3.4 per cent.; glycocol, 0.1 per cent., in distilled water.

[5] Behring: "Geschichte der Diphtherie," Leipzig, 1893.

Under certain conditions the human throat affords a lodging-place where the diphtheria bacillus is abundantly supplied with food, where it finds a highly suitable temperature for its growth and toxin-production, and where it is perhaps also benefited by the current of warm, moist air passing over the surface. That toxin is formed in the false membrane, whence it passes into the underlying tissues and diffuses through the body, causing injury to certain tissue cells for which it possesses a special chemical affinity, is a supposition quite in accord with all the observed facts.

Diphtheria Antitoxin.—Behring and Kitasato,[1] in 1890, found that the serum of rabbits immunized against diphtheria and tetanus by inoculation first with attenuated, then with virulent, cultures, contained a substance capable of neutralizing the effects of infection or intoxication in other animals. The action was found to be specific. The practical importance of this discovery in the case of diphtheria was soon made evident by the further researches of Behring, Wernicke, Knorr, Roux and Martin, and others.[2] The active principle in the blood and blood-serum is still chemically unknown, but from its neutralizing power it has been designated the diphtheria antitoxin. Somewhat later it was found that small non-fatal doses of toxin injected into the susceptible body were as effective in producing antitoxin as inoculation with the bacilli themselves. The amount of antitoxin that appears in the blood increases up to a certain point in proportion to increasing doses of the toxin, but the physiologic capacities of each individual animal limit the total amount of antitoxin produced. The antitoxic substance persists in the blood or blood-serum for a considerable period after the blood is drawn. It has been shown that the serum obtained from an immunized animal may retain unimpaired for many months its power of neutralizing diphtheria toxin when properly protected against putrefaction and the action of light or of high temperature. The probable mode of action of the antitoxin, and other theoretical considerations, are treated in the chapter on Immunity (p. 153).

In the preparation of diphtheria antitoxin on a large scale certain procedures are generally followed. Horses have been found especially suited for antitoxin production, both on account of their

[1] Behring and Kitasato: Deut. med. Wchnschr., 1890, 16, pp. 1113, 1145; also Behring: "Die Blutserumtherapie," Berlin, 1902.

[2] For references to these see Roux and Martin: Ann. de l'Inst. Past., 1894, 8, p. 609.

size and their relative endurance to the treatment with toxin. As a rule, gradually increasing quantities of toxin are injected into the subcutaneous tissue of the horse at intervals of five to seven days during a period of about two or three months. Not all animals prove equally tolerant of the treatment or yield a satisfactory quantity of antitoxin, and a continued selection of the particularly well adapted animals goes on in every large establishment. When the antitoxin in the blood reaches a desirable potency, for example, if the blood contains over 300 units per cubic centimeter, blood is drawn from the jugular vein into sterile glass jars and allowed to clot; from five to eight liters may be drawn at a time without injury to the animal, and bleeding may be repeated as often as once a month. The serum that separates from the clot is drawn off aseptically. It is then usually filtered through a Berkefeld filter, protected against contamination by the addition of carbolic acid, chloroform, or tricresol, tested and standardized, and bottled or placed in syringes as the diphtheria antitoxin of commerce. If diphtheria antitoxin is kept in the dark and at a low temperature, it loses strength very gradually. Some sera show hardly any decrease in potency during periods of a year or more. Those sera containing the largest number of units per cubic centimeter are more apt to lose strength than the so-called low-potency sera. A powdery deposit often forms in antitoxic sera after a time, but does not impair their value. Diphtheria antitoxin may be concentrated to some degree by precipitating, redissolving, and dialyzing.

The Concentration of Diphtheria Antitoxin.—Two chief methods of concentrating diphtheria antitoxin are in use in various laboratories. The first method is based on precipitation by means of ammonium sulfate, the second on precipitation by moderate heat and ammonium sulfate precipitation. The former method for precipitation of diphtheria antitoxin was worked out by Gibson.[1] The serum is precipitated by adding the same volume of a saturated solution of chemically pure ammonium sulfate. The precipitate formed is allowed to stand for about ten to twelve hours and then collected on large folded filters, preferably of hard paper. The precipitate is redissolved in a sufficient quantity of water to make up the volume of the original serum. After solution of the precipitate a quantity of saturated ammonium sulfate solution equal to the

[1] Gibson: Jour. Biol. Chem., 1905, 1, p. 161.

amount of water used for solution is added, and the precipitate which is formed collected in the same manner as the first precipitate. The precipitate is dried by pressure between mats of filter-paper, and when of about the consistency of putty is dissolved in a saturated solution of sodium chlorid. The solution is filtered, and the filtrate, which contains the antitoxin, is precipitated by adding 2.5 c.c. acetic acid (99.5 per cent.) to each liter, and the precipitate collected on hard folded filters. This precipitate is again dried between filter paper and then dialyzed in heavy parchment paper against running water until dissolved. After solution the fluid is neutralized to litmus paper with a solution of Na_2CO_3, returned to the dialyzing bag, and dialyzed until the sodium chlorid has been practically removed.

The principle of this method of concentration is the separation of the pseudoglobulins which contain the antitoxic principle from the other constituents of the blood-serum. The precipitation with ammonium sulfate removes the albumins by leaving them in solution. The second precipitation is for the purpose of removing the remnants of albumin. Saturated sodium chlorid solution dissolves the pseudoglobulin fraction, but not the other globulin fractions, so that the euglobulin and fibrinoglobulin are removed by filtration. Acetic acid in small amounts precipitates the pseudoglobulins in saturated sodium chlorid solutions.

This method was improved by Banzhaf and Gibson,[1] who employed a fractional method of precipitation. The serum is first diluted with the same volume of water, and to this diluted serum is added half its volume of saturated ammonium sulfate solution. The precipitate which is formed is collected on hard folded filters, pressed nearly dry, and dissolved in saturated sodium chlorid solution, the solution being treated with acetic acid, and the resulting precipitate dialyzed in the same manner as the previous method. To the filtrate of the ammonium sulfate precipitate is added enough ammonium sulfate solution to bring the percentage up to about 38. The precipitate is then treated exactly like the previous one. To the remaining filtrate, ammonium sulfate solution is added again until the saturation is 50 per cent. and the precipitate collected in the same manner. Three fractions of antitoxin globulins are obtained by this method. The last fraction

[1] Banzhaf and Gibson: Jour. Biol. Chem., 1907, 3, p. 253.

contains the largest amount of antitoxin and may yield a concentration considerably higher than by the original method of Gibson. The first and second precipitates contain but small amounts of antitoxin.

An entirely new method of concentration was worked out by Banzhaf.[1] The serum is diluted with one-half its volume of water, and to each 7 c.c. of diluted serum are added 3 c.c. of saturated ammonium sulfate solution. For two hours this mixture is heated in a water-bath until it gradually reaches a temperature of 60° C., where it is held for fifteen minutes, and then filtered while hot. To the filtrate is added enough saturated ammonium sulfate solution to bring the saturation to 50 per cent. The precipitate is gathered on hard filters, pressed and dialyzed. By heating with 30 per cent. ammonium sulfate solution some of the globulins are altered so as to render the pseudoglobulin fraction smaller, while retaining all the antitoxin. It is claimed that by this method a concentration of ten times the original potency can occasionally be obtained, while by the original Gibson method the concentration is two and one-half to three times, and by the fractional method three to five times.

Concentrated sera have several advantages over native sera. The amount to be injected is smaller, an advantage of importance when large or frequent doses have to be given. Some of the superfluous constituents of blood-serum are eliminated, constituents which may aid in the production of rashes or urticaria. One advantage is the greater yield made possible by the concentration, since with this method it is feasible to draw the blood from the horses into a solution of sodium citrate or, better, into a solution of potassium oxalate. Coagulation is prevented by the use of these chemicals, and a high yield of plasma can be obtained. The fibrinogen which remains dissolved in the oxalate plasma is precipitated with the first precipitate by the fractional method and by the heat in the last-mentioned method of concentration.

The globulin solutions after dialysis are filtered by suction through paper pulp in a Buchner funnel until perfectly clear, and then, after addition of 0.3 per cent. tricresol, are passed by suction or positive pressure (Fig. 10) through Berkefeld filters into sterile bottles.

[1] Banzhaf: Bull. of the New York City Antitoxin Laboratory, 1909.

The Curative Value of Diphtheria Antitoxin.—In order to cure a case of diphtheria in man, either the crude or the concentrated diphtheria antitoxin is injected with a sterilized syringe into the loose subcutaneous tissue, the best locality being in the loose tissue of the loin (just below the short ribs). Injection may be made in the back near the angle of the scapula, but is more painful.

Recent studies indicate that the entire dose deemed necessary should be injected at the first treatment.[1] Park advises in mild cases 3000 to 5000 units, in moderate cases 10,000 units and in severe toxic cases 20,000 units or more in adults, and 10,000 to 20,000 units or more in children. In the very serious cases intravenous injection is to be preferred to subcutaneous. Administration of antitoxin by the mouth is valueless. There is no limit, as far as the antitoxin is concerned, to the number of units that may be safely injected.

Many attempts have been made to lessen the amount of fluid containing a given number of units. The use of a powerful toxin for immunization, the selection of horses that yield a particularly large number of antitoxin units per cubic centimeter, and improvements in methods of immunization and concentration have resulted in lowering considerably the dose of horse-serum. Serum containing 500 to 700 units per cubic centimeter is now commonly marketed, and a few horses have been known to yield serum of 1000 to 1500 unit strength. The so-called low-potency sera, however, containing about 300 to 500 units per cubic centimeter, are, unit for unit, just as efficacious, and can be produced much more economically than the high-potency sera.

The administration of antitoxin is followed in some cases by temporary pain in the joints and by rashes. The rashes appear to be due to unknown substances in the horse-serum, which are present in larger amounts in some horses than in others. These substances may be present in the blood of a horse at one bleeding and absent at the next. They occur in both normal and immune animals. The concentrated serum contains, as a rule, less of these rash-producing substances than the original serum.

A few cases of sudden death have been reported following the administration of antitoxin serum.[2] The cause is unknown. It is

[1] Park: Trans. Assoc. Amer. Phys., 1912, 27, p. 434.

[2] See, for example, Jour. Amer. Med. Assoc., 1908, 50, pp. 137, 456, and 468.

possible that sensitization of the organism to horse-serum (see p. 190) is responsible, or that some obscure individual peculiarity lies at the bottom of the trouble. In comparison with the enormous number of antitoxin injections constantly made, such cases appear to be exceedingly rare.

The Results of Antitoxin Treatment.—The efficacy of antitoxin treatment is matter of common knowledge and the results need only be outlined here. The death-rate from diphtheria in certain large American cities before and after antitoxin came into use (1894–95) is considered to illustrate the saving of life that has been effected.

AVERAGE ANNUAL DEATH RATE FROM DIPHTHERIA IN 10 LARGEST CITIES IN THE UNITED STATES PER 100,000 POPULATION

	1890–94.	1920–24.
New York	134.4	14.0
Chicago	117.3	17.5
Philadelphia	119.4	16.7
Detroit	132.9	24.3
Cleveland	95.7	14.7
St. Louis	67.7	16.1
Boston	112.2	20.2
Baltimore	70.0	11.4
Pittsburgh	86.4	20.1
Los Angeles	46.0	14.4

In 1890–94 fifty large American cities had diphtheria death rates above 40 per 100,000 and only two had rates under 10; in 1926 no city had a rate above 35 and fifty-seven had a rate under 10. It must be remembered that, while the greater part of this reduction in diphtheria is undoubtedly due to antitoxin, part of it may be attributed to the mildness of the type of diphtheria that has prevailed in recent years.

Modes of Infection.—The source of infection is the human carrier of diphtheria bacilli. The ordinary way in which diphtheria is spread is by more or less direct transfer of bacilli from a mild or severe case of the disease or from a convalescent patient or from a well person that has come into contact with a case of diphtheria and harbors the specific germ in his throat or nose. It has already been pointed out that diphtheria bacilli may retain their virulence for a long time in particles of dried membrane. The germs have been found on children's toys, in the dust of sick-rooms, and clinging to the clothing of nurses. Under favorable conditions

they may resist drying for several weeks. The chief danger, however, apparently does not depend upon dissemination of dust particles or upon inadequate disinfection of the surroundings of a patient, but rather upon the patient or convalescent himself. For many days, and exceptionally for months, after complete recovery the bacilli may persist in the throat and nose. Fully virulent germs have been found in a child's throat for as long as 335 days after the cessation of clinical manifestations. When diphtheria is prevalent in a school or an institution, bacilli are frequently found in a large percentage of the throats of perfectly healthy children. There is abundant evidence, therefore, that healthy individuals are sometimes the carriers of virulent diphtheria germs, and may be the means of causing serious epidemics. Chronic membranous rhinitis, due to the diphtheria bacillus, is not infrequently a source of infection. Bacilli isolated from an apparently healthy carrier have been experimentally introduced into the throat of adult human volunteers, a procedure promptly followed by clinical diphtheria in several instances. Just what proportion of diphtheria cases is actually caused by contact with carriers as distinguished from contact with active cases is not known. Certain investigations in Baltimore indicated that the risk of developing clinical diphtheria through contact with "healthy carriers" may not be so great as at one time believed.[1]

It is well known that the disease is much more prevalent, as well as more fatal, among children than among adults. The effect of school attendance upon the spread of diphtheria has been clearly shown by English health officials.[2] Not only has an increase in the prevalence of the disease in England been noticed coincident with the putting into operation of a compulsory education act (1870), but the number of cases of diphtheria reported has been observed to rise and fall in direct sequence to the occurrence of holidays and the resumption of school work. "The relation between school closure and diphtheria prevalence in Chelsea (Eng.) is shown in the following table, in which are set out the average number of cases occurring at all ages, and at the school age of three to thirteen years, in thirteen four-weekly periods, during the five non-epidemic years, 1890–1894 inclusive[3]:

[1] Doull and Lara: Am. Jour. Hyg., 1925, 5, p. 508.
[2] Murphy: Lancet, 1894, 2, pp. 1403, 1409.
[3] Parkes: Trans. Epidemiol. Soc., London, 1896, N. S. 16, p. 240.

"DIPHTHERIA IN CHELSEA

Weeks.	Total Cases.	Cases at Ages Three to Thirteen.
1–4	**8.0**	**3.8**
5–8	11.4	4.8
9–12	10.8	5.2
13–16	11.4	5.6
17–20	13.6	5.6
21–24	11.8	5.4
25–28	16.4	7.4
29–32	14.8	8.2
33–36	**9.6**	**3.0**
37–40	14.2	7.0
41–44	13.8	7.8
45–48	21.2	11.8
49–52	15.0	8.8"

The closure of the schools for the summer vacation (lasting a month), which usually commences in the thirtieth week of the year, is followed by a fall in the number of notified cases (thirty-third to thirty-sixth week). There is a corresponding fall after the Christmas holidays (first four weeks).

The use of the common drinking-cup and moistened lead-pencil, the friendly transfer of pocket-handkerchiefs, candy and chewing-gum, and the other familiar practices of school-children, afford opportunities for an immediate and tolerably direct passage of bacilli from an infected individual to a healthy one. It is probable, too, that in the act of coughing or talking droplets of moisture or mucus containing bacilli pass into the air and may be inhaled by bystanders. The most usual mode of infection, however, is direct contact. There is no evidence that diphtheria bacilli are present in ordinary sewer air or that they ever effect an entrance to a dwelling through defective plumbing. It must be pointed out, however, that any condition, such as a damp or "raw" atmosphere, which tends to produce an irritated or weakened mucous membrane, is distinctly favorable to infection.

It is well established that diphtheria is sometimes disseminated by infected milk. Certain domestic pets, notably cats, have been popularly regarded as being able to communicate the disease,[1] but experiments have shown that the implantation of vast numbers of diphtheria bacilli into the nasal cavities of cats has failed to set up any general or local lesions and the bacilli have quickly dis-

[1] Cf. Thorne: "Diphtheria," London, 1891.

appeared.[1] There is no ground for believing that cats can serve as carriers of diphtheritic infection.

The so-called *avian diphtheria*, or "roup" of fowls and pigeons, has been frequently asserted to be due to the same micro-organism as that causing human diphtheria, but there are insurmountable objections to this view. The most important of these is the fact that the antitoxin which protects against the Klebs-Löffler bacillus is without effect upon the progress of roup. Moreover, the bacillus usually present in avian diphtheria has been isolated and differs in essential particulars from the Klebs-Löffler bacillus.

Mixed Infections.—The Klebs-Löffler bacillus is found almost invariably associated in the false membrane with streptococci, staphylococci, or other micro-organisms. There is no doubt that these bacteria, especially the streptococci, often play an important part in both the local and general development of the infection. Certain common complications of diphtheria, particularly suppurative processes in the tissues of the neck, are unquestionably due to the action of the associated pyogenic organisms. Vincent's angina (p. 504) is a dangerous complication of diphtheria. Some observers have claimed that the virulence of the diphtheria bacilli is increased by symbiosis with the streptococci, but there is no convincing evidence in favor of this view. There have been a number of attempts to gage the influence of these mixed infections on the outcome of a diphtheritic attack. Simple streptococcus anginas are unquestionably of a more benign character than throat affections in which the Klebs-Löffler bacillus is found, either as the sole or predominant organism; but regarding the mixed infections of streptococci and diphtheria bacilli, opinions have been at variance. From analogies observed in animal experimentation, as well as on other grounds, there is reason to believe that the mixed infections, in which the streptococcus is actually sharing in the production of pathologic processes, are of a more serious character than those in which the Klebs-Löffler bacillus alone is the active factor.

Diphtheroid Bacilli.—Bacteria with the morphological characters of the diphtheria bacillus have been supposed on more or less plausible grounds to be causally related to certain infections. Xerosis (p. 281) and Hodgkin's Disease (p. 721) are mentioned elsewhere.

[1] Savage: Jour. Hyg., 1920, 18, p. 448.

The skin eruption known as *acne* appears to be caused by a diphtheroid bacillus (B. acne) which grows in the presence of only a small amount of oxygen. Its growth in most artificial culture media is at best rather feeble. Specific vaccines made with this organism are thought to have a beneficial effect.

Bacteria that closely resemble B. diphtheriæ but are relatively less virulent are not uncommonly encountered in the throats and noses of healthy individuals, in the conjunctival sac, and even in the false membrane in typical clinical diphtheria side by side with the true diphtheria bacillus. Löffler,[1] in 1887, reported finding an organism of this character in a diphtheritic membrane, and general attention was soon directed to the significance of the so-called pseudo-diphtheria bacilli by the similar observations of v. Hofmann-Wellenhof.[1] This organism, often called by the name *Hofmann's bacillus*, grows more luxuriantly upon agar than the Klebs-Löffler bacillus, is somewhat shorter and plumper, does not show granules when treated by the Neisser stain and fails to produce acid in dextrose broth. It is, moreover, nonvirulent for guinea-pigs. While it is relatively easy to separate typical pseudo-diphtheria bacilli possessing these qualities from B. diphtheriæ, considerable difficulty has arisen in attempting to apply in all cases a strict criterion of differentiation. Now and again bacilli are found that resemble B. diphtheriæ in all important respects except in their lack of virulence, and by some authors these are classed as pseudo-diphtheria bacilli, although this is not in accordance with general usage. Such forms on closer study are often found to produce a small amount of toxin and are more properly regarded as attenuated forms. On the other hand, virulent bacilli have sometimes been found that do not ferment dextrose, but these also, owing to their specific toxin production, must be regarded as genuine examples of B. diphtheriæ. Neisser's strain is now generally admitted to be a far from absolute criterion. A virulence is generally, although

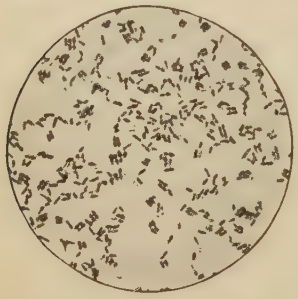


Fig. 61.—Diphtheroid bacilli, methylene-blue; × 1000 (Park).

[1] Löffler: Centralbl. f. Bakt., 1887, 2, p. 105.

[2] Von Hofmann-Wellenhof: Wien. med. Wchnschr., 1888, 38, pp. 65, 108.

not invariably, associated with inability to ferment dextrose and with the absence of granules.

There are not wanting statements by several observers to the effect that a transformation of one form into the other has been accomplished, and there are some who believe that the pseudo-diphtheria bacillus or the avirulent variety is simply a modified form of B. diphtheriæ, which has parted with its virulence and certain other properties under the stress of altered conditions. Statements by Lesieur,[1] for example, are especially specific on the question of transformation. It is claimed that virulent cultures of B. diphtheriæ have been transformed by the action of daylight into non-virulent ones exhibiting all the characters of the Hofmann bacillus, and, reciprocally, pseudo-diphtheritic bacilli have, by cultivation in collodion sacs within the bodies of rabbits, been transformed into organisms possessed of the qualities distinguishing B. diphtheriæ.

Perhaps the most definite statements about transformation are those made by Cromwell,[2] who in studying single cell strains under different conditions of cultivation found that non-toxic strains could be produced "by mutation" from toxic strains and remained permanently non-toxic. The relation of these non-toxic derivatives to the Hofmann bacillus and similar forms is still not altogether clear.

Morse[3] has divided the diphtheroid bacilli into four sub-groups. The fermentation reactions of these sub-groups are as follows:

Group.	Dextrose.	Sucrose.	Maltose.	Glycerol.
A (B. hoagii)	+	+	−	−
B (B. flavidus)	+	−	±	±
C (B. xerosis)	+	±	±	+
D (B. hofmanni)	−	−	−	−

Organisms of Group A type have been most commonly reported; Group C is relatively rare.

Mellon,[4] as the result of a very comprehensive study of the characters of the diphtheroid group, regards the group as one of great diversity and lability and establishes three more sub-groups (B. diphtheroides liquefaciens, B. enzymicus, and B. ruedigeri) in addition to those already recognized by Morse. One of these, B.

[1] Lesieur: Jour. de Physiol. et de Path. gén., 1901, 3, pp. 961, 1000.
[2] Cromwell: Jour. Bact., 1926, 11, p. 65.
[3] Mary Morse: Jour. Infect. Dis., 1912, 11, p. 253.
[4] Mellon: Jour. Bact., 1917, 2, p. 269.

enzymicus, is believed by Mellon to be closely related to the streptococci. B. enzymicus, B. flavidus, and B. ruedigeri are considered the most pathogenic members of the group. In Mellon's experiments the most decisive group differentiation resulted from the use of the complement-fixation reaction.

From the public health point of view the question of the interrelation of the "real" and "pseudo" forms is of practical importance. It has been found that a considerable proportion of healthy persons in every community harbor so-called pseudo-diphtheria bacilli (16 to 22 per cent.), but whether or not these persons constitute a menace to the health of the community is a question upon which opinions differ. The same investigations[1] show that about 1 or 2 per cent. of all persons harbor typical Klebs-Löffler bacilli (of the granular type), but that in this number only about 17 per cent. have virulent bacilli, "or, in other words,17 in 5000 to 10,000 of all persons have diphtheria bacilli that are dangerous to the health." If it were true that the solid-staining, avirulent, pseudo-forms might suddenly, when transferred to a susceptible individual, acquire pathogenic power, the protection of the community by quarantine and isolation would present a different aspect. Further experimental study of well-defined diphtheria and pseudo-diphtheria bacilli is needed, especially in relation to virulence and variation.

In the light of our present knowledge the conclusion seems justified that there are at least two independent and distinct groups of organisms, the diphtheria (Klebs-Löffler) bacilli and the diphtheroid or pseudo-diphtheria bacilli. The true diphtheria bacillus is rarely found except in diphtheria patients (including latent cases of apparently simple "sore throat"), convalescents, and persons in contact with such cases. There does not seem to be adequate evidence of the transformation of non-virulent pseudo-diphtheria bacilli into the true toxin-producing type.

Among the true diphtheria bacilli different serological types have been distinguished.[2] Havens recognized two; Park, Williams, and Mann,[3] five, and J. Smith,[4] seven. Powell,[5] working with single-

[1] Jour. Mass. Assn. of Boards of Health, 1902, 12, p. 75.
[2] Havens: Jour. Infect. Dis., 1920, 26, p. 388.
[3] Park, Williams, and Mann: J. Immunol., 1922, 7, p. 273.
[4] J. Smith: Jour. Hyg., 1923, 22, p. 1.
[5] Powell: Amer. Jour. Hyg., 1923, 3, p. 362.

cell cultures, found considerable stability in the agglutination reactions; his cultures, five virulent, ten avirulent, fell into eight groups.

Method of Diagnosis.—The examination of suspected throats for the diphtheria bacillus has been for years part of the routine work of a number of large and well-managed municipal laboratories in this country, and the methods employed after careful investigation have become well crystallized.[1] Outfits for this work are issued to physicians on request. These consist, as a rule, of a tube of Löffler's serum, which should be freshly prepared, a tube containing one or more sterile swabs, and printed directions and record forms. The whole outfit is inclosed in a metal or pasteboard box. The swabs are usually composed of pledgets of sterile cotton wound about the end of a wire or piece of wood. Some boards of health require that separate cultures be taken from the nose and throat when the examination is being made for the purpose of determining the end of a prescribed isolation period. Sometimes a cover-slip film is made at once from the swab for immediate microscopic examination, but this procedure, although it sometimes facilitates a speedy diagnosis, will often give a negative result if bacilli are present in small numbers. The method of cultivation is much more reliable. After the swab has been rubbed carefully over the serum, the tube is incubated at 37° C. until the next morning, when cover-slip films are made from the growth on the medium and examined microscopically in the usual way. A single negative result or even more cannot be taken as conclusive proof that diphtheria bacilli were actually absent in the throat at the time the swab was used, since if only a few bacilli are present, or if they are in relatively inaccessible locations, as in laryngeal cases, they might easily be missed. Many boards of health require two or more successive negative cultures for release from quarantine.

Serum Prophylaxis.—In addition to the ordinary measures of quarantine and isolation, which, to be effective, should be based upon the systematic bacterial examination of the throat and nose of convalescents, a number of other methods have been suggested for checking the dissemination of the disease. It has been shown that diphtheria is primarily a disease of school-children and is largely

[1] See Report of Committee on Throat Cultures, Amer. Med. Assoc., Jour. Amer. Med. Assoc., 1911, 57, p. 976.

affected by school attendance. An appreciation of this fact by the school authorities and the introduction of methods of examination and prompt isolation have led in some quarters to a material reduction of contagion. It should be clearly recognized that the disease is kept smouldering in the community and fresh outbreaks lighted up chiefly by infected individuals who mingle with their fellows. Experience has shown that the throats of persons coming in contact with a case of diphtheria are quite likely to harbor diphtheria bacilli, and that these persons, although themselves remaining well, may become centers for the further spread of this disease. The use of the antitoxic serum in doses of 500 or, better, 1000 units has been widely advocated as a prophylactic measure. A considerable degree of protection can be conferred in this way upon the children in a family where a case of diphtheria has appeared, and upon nurses or other attendants. Records of the Health Department of the City of Baltimore[1] show that only one case of diphtheria developed among three hundred and eighty-two children who, after more or less exposure, had been given 1000-unit doses. In certain European hospitals a preventive dose of antitoxin is given at regular intervals as a matter of routine to all children in the institution, and this procedure is said to be attended with great success. The passive immunity so obtained is relatively transient; it begins about twenty-four hours after the injection and is practically at an end after twenty-eight days.

The Schick Reaction.—A method of determining the susceptibility of a person to diphtheria was devised by Schick in 1913.[2] The reaction depends on the fact that a local irritation is caused by a minute quantity of diphtheria toxin injected into the skin of a susceptible person, while if the person is immune the skin reaction does not occur. Many individuals are naturally resistant to diphtheria, and this method affords a means of selecting for artificial immunization only those in need of protection. The amount of toxin injected for the Schick test is 0.2 c.c. of normal saline containing $\frac{1}{50}$ of the minimal lethal dose (M. L. D.) for a guinea-pig weighing 250 to 300 grams. A negative reaction may be taken to indicate that one c.c. of the individual's serum contains at least $\frac{1}{20}$ unit of diphtheria antitoxin and that he is probably safe against infection;

[1] Annual Report, 1904, p. 86.

[2] Schick: Münch. med. Wchnschr., 1913, 60, p. 2608.

a positive reaction indicates the presence of less than $\frac{1}{10}$ antitoxin unit per c.c. and points to the necessity for an immunization in the event of exposure to infection.

The practical application of the Schick reaction has been especially studied in the laboratories of the New York City Health Department.[1] The percentage of individuals susceptible to diphtheria is shown to be greatest between the ages of one and five years.

SUSCEPTIBILITY OF VARIOUS AGES TO DIPHTHERIA

(As Indicated by Diphtheria-Toxin Skin Test)

Age.	Susceptible.
Under three months	15 per cent.
Three to six months	30 "
Six months to one year	60 "
One to two years	70 "
Two to three years	60 "
Three to five years	40 "
Five to ten years	30 "
Ten to twenty years	20 "
Over twenty years	12 "

Natural immunity when it once develops in a child seems to be a lifelong possession.

The Schick test needs to be made by an experienced observer since there are certain difficulties and sources of error that complicate the practical application. In older children and adults a "pseudo-reaction" may occur due to the protein in the material injected and having nothing to do with the specific toxin. Confusion from the pseudo-reaction may be avoided by injecting the toxin in the skin of one arm and the same amount of heated or antitoxin-neutralized toxin in the other arm.

When the susceptible children in a school or institution have been discovered by the application of the Schick test the question of the best method of immunization must be considered. Passive immunization with diphtheria antitoxin has the disadvantage that it is very transient. Active immunization with harmless toxin-antitoxin mixtures has therefore come into quite general use. It is based on observations by Behring and by Theobald Smith. Excellent results have been obtained by this method.

Mixtures containing about 2L+ doses of toxin per cubic centimeter have been used; these are rendered either neutral or slightly

[1] Park: Monthly Bull., Dept. of Health, New York City, 1919, 9, p. 65.

toxic to the guinea-pig (66 to 90 per cent. L+ to each unit of antitoxin). From one to three subcutaneous injections of 0.5 to 1 c.c. are used at intervals of seven days. Strict care must of course be exercised in the proper adjustment of the mixture. Toxin detoxified with chemicals such as sodium ricinoleate has been used by some workers in place of the toxin-antitoxin mixtures.

The reaction caused by the injection of the toxin-antitoxin mixture is usually slight, and the immunity conferred lasts in 90 per cent. for more than six years and probably much longer. The New York observers conclude that it is advisable to immunize children soon after the first year of life in order to afford protection at the ages when the disease is most dangerous.

The possibilities of the Schick test followed by toxin-antitoxin immunization are shown by the experience of the State Industrial School for Boys at Shirley, Mass.[1] Of the inmates, 257 in number, 148 gave positive Schick reactions. These susceptible individuals were then given three doses of the toxin-antitoxin mixture. Following this procedure every new boy entering the institution was tested for his susceptibility, and if this was positive he was immunized. By practising this method for two years diphtheria was completely eliminated from the school—a result which had not been obtained previously when other methods were employed. In several American cities in which toxin-antitoxin immunization has been largely practiced diphtheria has been greatly reduced. During the five-year period 1922–1926, the average number of cases in New York City dropped 34 per cent. as compared with the previous five years 1917–1921.[2]

[1] T. E. Lilly: Public Health Bull., Dept of Health, Mass., May, 1918.

[2] See also Scammon and Pope: "Diphtheria Immunization in Providence," Jour. Am. Med. Assn., 1927, 88, p. 563.

CHAPTER XVI

THE GROUP OF COLON-TYPHOID BACILLI

Plump rods with rounded ends and flagella. More or less motile. No spores. Gram-negative.

Three groups: B. coli; paratyphoid-enteritidis; B. typhosus, B. dysenteriæ, Alcaligenes fecalis.

One of the most important groups of pathogenic bacilli is that which includes the typhoid bacillus, the bacillus of dysentery, certain bacilli found in cases of paratyphoid fever and in food-poisoning, and other related organisms. The group is a large one and comprises many varieties that are as yet imperfectly separated from one another. Great advances, however, have been made in differentiation within this group in the last few years.

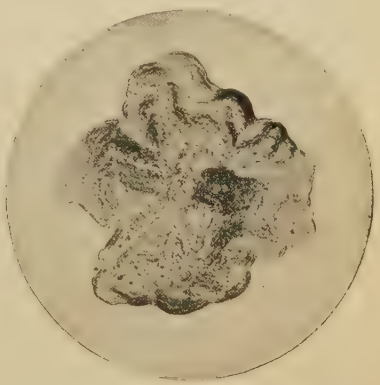


Fig. 62.—Bacillus typhosus colony on gelatin, seventy-two hours old; unstained; Neufeld prep. (Kolle and Wassermann).

Characteristics and Subdivisions of the Group.—Under favorable conditions of growth the prevailing form is a plump, straight rod with rounded ends. Short, oval forms are not uncommon upon certain media, and long filamentous forms are occasionally developed, especially at high temperatures. The cell protoplasm often stains irregularly when carbol-fuchsin is used. The bacilli lose the stain when treated by Gram's method. No spores are known to be formed by any of this group.

One of the distinguishing cultural characteristics of these organisms is their mode of growth upon the surface of gelatin. This feature is well seen in colonies upon a gelatin plate. Upon the surface of the medium an irregular, thin, notched, leaf-like expansion is formed, which is so typically produced by members

of the group that the term "colon-like" or "typhoid-like" has been applied to this kind of colony (Fig. 62). This constitutes one of the more constant distinguishing growth characters of the group although B. [lactis] aërogenes, an organism in most respects closely related to the colon bacillus, develops colonies that are projecting, have rounded margins, and depart materially from the type. In no case is gelatin liquefied. All the organisms of this group are able to reduce nitrate to nitrite in the presence of organic matter, and the majority of strains are able also to reduce nitrite with evolution of nitrogen.

A particularly interesting biologic character of the group is that many of the members appear to be on the verge of parasitism, and, as it were, stand ready to assume a parasitic mode of life when a favorable opportunity presents itself. Many varieties, such as the forms found in the intestines of certain of the higher animals, occupy a position intermediate between saprophytes and parasites, and from this point of view the group is at present in a state of unstable biologic equilibrium and must be looked upon as possessing marked potentialities for evolution in the direction of parasitism.

Three subdivisions of the group can at present be distinguished:

1. B. coli; the capsulated bacteria.

2. The paratyphoid-enteritidis group, including B. suipestifer, B. enteritidis, B. paratyphosus A and B, B. psittacosis, and various other "paracolon" and "paratyphoid" bacilli.

3. B. typhosus and B. dysenteriæ; Alcaligenes fecalis.

Some of the main points of difference between the several subdivisions are shown in the following comparison:

1. *B. coli:* Motility is not pronounced in most cultures; often only a few individuals in the field show independent movement; few flagella. Dextrose and lactose are fermented with gas-production, some varieties fermenting saccharose also. Milk is curdled, usually within forty-eight hours, at 37°, with abundant acid production. Luxuriant growth on potato, usually with a brown tinge. Indol is produced by most varieties in large amount. Under most conditions not pathogenic for man or the lower animals. When injected intraperitoneally into rabbits and guinea-pigs a fatal result usually ensues, but considerable variation in virulence is shown by different strains.

2. *B. enteritidis:* Actively motile; 10 to 14 flagella. Dextrose is fermented with gas-production, but no gas or acid is formed from lactose. Milk is never curdled; on prolonged cultivation under aërobic conditions the milk acquires a slight primary acidity, then becomes strongly alkaline, and finally the casein is dissolved. Indol is not produced. Distinctly pathogenic for many of the lower animals and for man.

3. *B. typhosus:* The typhoid bacillus is actively motile and possesses 10 to 14 flagella. B. dysenteriæ, otherwise closely related, is slightly motile or devoid of motility, according to some observers. Dextrose is fermented with production of acid, but gas is never produced; no acid is produced from lactose. Milk is never curdled, although a slight initial acidity develops, followed by a return to the original reaction; and alkaline reaction is produced in milk by some stains, but not to such an extent as in the preceding group. No indol is formed under ordinary conditions of cultivation. Pathogenic for man, less so for the lower animals.

The most useful differential tests for the individual members of the group are given in the following table:

THE COLON-TYPHOID-DYSENTERY GROUP

Gram-negative bacilli ferment the hexoses[1]; are generally Voges-Proskauer − and methyl red +[2]; do not form spores; do not liquefy gelatin.[3]

Organism.	Indol.	Lead acetate.	Milk.	Gas production.	Maltose.	Mannitol.	Xylose.	Arabinose.	Rhamnose.	Sorbitol.	Dulcitol.	Lactose.	Salicin	Sucrose	Raffinose	Inositol	Trehalose.
Bacillus aerogenes	−	−	ac	+	+	+	+	+	+	+	∓	+	+	+	+	+	+
Bacillus cloacæ	∓	−	ac	+	+	+	+	+	+	+	∓	+	+	+	+	+*	+
Bacillus mucosus capsulatus	−	−	a	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Bacillus neapolitanus	+	−	ac	+	+	+	+	+	+	+	∓	+	+	+	+	−	
Bacillus coli communior	+	−	ac	+	+	+	+	+	+	+	∓	+	−	+	+	−	+
Bacillus coli communis	+	−	ac	+	+	+	∓	+	+	+	∓	+	+	−	∓	−	+
Bacillus anatum	−	+	ak	+	+	+	∓	+	+	+	+	−	−	−	−	+	+
Bacillus acidi-lactici	+	−	ac	+	+	+	+	+	+	+	∓	+	−	−	∓	−	+
Bacillus schottmülleri	−	+	ak	+	+	+	+	+	+	+	+	−	−	−	−	∓*	+
Bacillus enteritidis	−	+	ak	+	+	+	+	+	+	+	+	−	−	−	−	−	+
Bacillus abortivo-equinus	−	−	ak	+	+	+	+	+	+	+	+	−	−	−	−	−	+
Bacillus suipestifer	−	∓	ak	+	+	+	+	−	+	+	∓	−	−	−	−	−	−
Bacillus gallinarum	−	−	ak	−	+	+	+	+	+	+	+	−	−	−	−	−	+
Bacillus paratyphosus A	−	−	ak	+	+	+	−	+	+	+	+	−	−	−	−	−	+
Bacillus pullorum	−	−	ak	−	−	+	−	+	+	+	−	−	−	−	−	−	−
Bacillus typhosus	−	+	ak	−	+	+	+	−	−	+	−	−	−	−	−	−	+
Bacillus dysenteriæ (Flexner)	+	−	ak	−	∓	+	∓	∓	∓	∓	∓	−	∓	∓	∓	∓	+
Bacillus dysenteriæ (Shiga)	−	−	ak	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Bacillus fecalis alcaligenes	−	+	k	−	−	−	−	−	−	−	−	−	−	−	−	−	−

+ = feremention or positive test; − = negative test; ∓ = variable. "Gas production" + or − applies to fermentations. +* = acid, but no gas. a = acid; c = coagulation; k = alkali.

[1] B. fecalis alcaligenes excepted.

[2] B. aerogenes and B. cloacæ are V. P. + and m. r. −; B. dysenteriæ (Shiga) and B. fecalis alcaligenes are negative to both tests.

[3] B. cloacæ excepted.

Subdivision 1

Bacillus coli

The organism taken as the type of this class was described under the name of Bacterium coli commune by Escherich in 1886.[1] The original culture was isolated from the dejecta of a breast-fed infant, and cultures from this source are still considered by Escherich to be

[1] Escherich: "Die Darmbacterien des Säuglings," Stuttgart, 1886.

especially typical. Other bacteria which were discovered about the same time and variously designated, such as the "Naples cholera germ," or B. neapolitanus,[1] isolated by Emmerich[2] from the dejecta of patients suffering from Asiatic cholera, should doubtless rank as members of this class. The typical Bacillus coli is widely distributed in nature and has been isolated from air, from water, and from soil. In only a limited sense, however, is it "ubiquitous." It is found by far most abundantly and constantly in the intestinal tract of man and many of the higher animals. In the colon it occurs in especial abundance, and is so characteristic an inhabitant of this region of the intestine as fully to deserve the name that has been bestowed upon it. From fresh, healthy human feces it is often isolated in pure culture by the ordinary aërobic methods, although microscopic examination shows that other kinds of micro-organisms are also present in the feces. The varieties of B. coli that are isolated from the intestinal contents of different species of mammals differ slightly in their biologic characters. Moore,[3] for example, has shown that cultures of B. coli obtained from the intestines of the dog are more virulent, when injected intraperitoneally into guinea-pigs, than those from the intestine of the rabbit or guinea-pig.

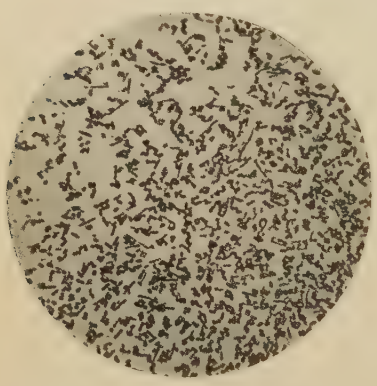


Fig. 63.—Bacillus coli; twenty-four-hour agar culture; × 650 (Heim).

Morphology.—The morphology of the colon bacillus exhibits considerable variation. The ordinary dimensions in stained preparations from cultures upon nutrient agar or gelatin range from 2 μ to 4 μ in length and from 0.4 μ to 0.7 μ in breadth (Fig. 63). Very short, oval and coccus-like forms are encountered not infrequently, and usually predominate when the bacillus is observed directly in normal animal tissues.

[1] According to a strict application of the rules of priority, the bacillus now known as B. coli should be called B. neapolitanus.

[2] Emmerich: Arch. f. Hyg., 1885, 3, p. 291.

[3] Moore: Amer. Med., 1902, 3, p. 504.

The most typical members of this group possess motility; flagella can be demonstrated by appropriate stains (Fig. 64).

Cultural Characteristics.—The more salient biologic characters of this subdivision of the colon-typhoid group have already been noted in tabular form. Gelatin is not liquefied; milk is curdled with acid reaction, usually within forty-eight hours, by the cultures that must be regarded as the more typical or more vigorous. Indol is produced in abundance by all vigorous strains. Dextrose is fermented, with the production of gas. Lactose, like dextrose, is always fermented with gas production by true colon bacilli.

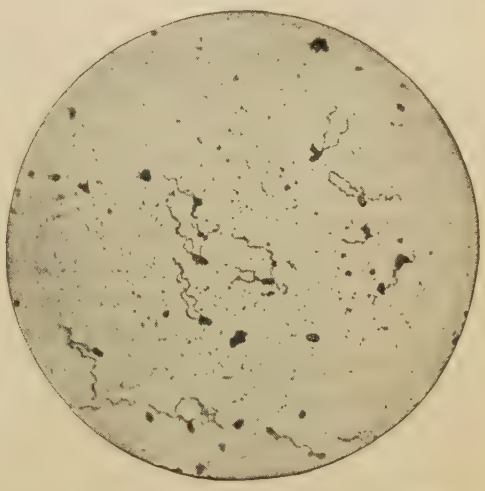


Fig. 64.—Bacillus coli with flagella stained by van Ermengem's method; × 1000 (Williams).

Rogers, Clark, and Davis[1] found by careful measurement of gas production in dextrose media with colon bacilli obtained from milk that the most frequent carbon dioxid to hydrogen ratio was approximately 1 : 1. Cultures giving this ratio, which they term the "low ratio," ferment a smaller number of test substances (adonite, starch, saccharose, raffinose) than those giving a large percentage of CO_2, the "high ratio" group. Colon bacilli from bovine feces yielded only 1 high ratio culture to 149 low ratio cultures. In milk the two groups were about equal in number.

Nearly one-half of the colon bacilli that are isolated from various sources produce gas in saccharose broth; they are not known to differ in other characters from those unable to ferment saccharose.[2] Attempts have been made by some writers to distinguish varieties of colon bacilli on the basis of differences in fermentive power. One of the distinctions made most frequently relates to differences in

[1] Rogers, Clark, and Davis: Jour. Infect. Dis., 1914, 14, p. 411.
[2] Jordan, Jour. of Hyg., 1903, 3, p. 1.

saccharose and dulcite fermentation. The names commonly used are as follows:

	Gas Produced from Saccharose.	Dulcite.
B. neapolitanus (B. coli communior)	+	+
B. coli (B. coli communis)	−	+
B. aërogenes[1]	+	−
B. acidi-lactici	−	−

No conclusions of value have yet been derived from studies of the distribution of these varieties in normal or diseased conditions or in polluted waters.

Certain bacilli not infrequently isolated from water, soil, and other sources, are regarded by many investigators as weakened or aberrant members of the colon group. They depart from the so-called type in several more or less significant respects. A not uncommon divergence consists in the inability to generate any considerable quantity of acid in milk, so that the milk is not curdled promptly (*i. e.*, within forty-eight hours at 37°), or in some cases not at all. Ability to produce indol in peptone solution is likewise lacking in some cultures otherwise typical. Certain of these bacilli are obviously attentuated forms of B. coli, and may be made to approximate closely to the type by the method of rejuvenation or broth cultivation elsewhere described (p. 59). A few observers would include in the colon group somewhat similar organisms that are unable to produce gas in dextrose broth, although in other respects possessing the biologic characters of the type; but it is not generally considered that forms devoid of this fermentive power should be classed as colon bacilli. When the colon bacillus is introduced into the bodies of various animals, it is able to bring about certain morbid changes, as shown by Emmerich in his study of "B. neapolitanus," and confirmed since by many workers. Intraperitoneal injection of 2 c.c. of a twenty-four- to forty-eight-hour-old broth culture usually proves fatal to a guinea-pig within three days. Cultures isolated from various tissue lesions and suppurative processes are more virulent for animals than those isolated from the normal intestine. Subcutaneous inoculation is much less likely to result fatally than intraperitoneal. Some writers assert that cultures of B. coli isolated from the contents of a diseased intestine are more virulent than those from a normal individual, but investigators are not in accord on this point.

[1] See also p. 313.

Pathogenicity.—As regards pathogenicity for man, the common occurrence of agonal or post-mortem invasion of the body by the colon bacillus tends to diminish the value of the supposed evidence derived from finding the colon bacillus in the internal organs after death, and there can be no doubt that the rôle in human pathology assigned to the colon bacillus by some investigators, notably certain French bacteriologists, has been greatly exaggerated. Failure to distinguish between the true colon group and the group of meat-poisoning bacilli is doubtless responsible for some of the statements attributing pronounced pathogenic properties to B. coli. The frequent ascription of various inflammatory processes, particularly those occurring in the appendix and peritoneum, to the unaided activities of B. coli appears to be without sufficient justification. Many of the cases reported rest on the evidence derived from simple aërobic cultivation, where the possible concurrence of anaërobes or other organisms not growing by ordinary methods has not been excluded.

It is well established, however, that under some conditions the colon bacillus is able to pass from the digestive tract into the blood, whence it may invade the gall-bladder and bile-ducts and cause cholangitis and cholecystitis. Convincing evidence on this score has been obtained both from human pathology and from animal experiment. The bacillus is often found in the core of gall-stones. Kramer[1] has made the interesting observation that in cultures the colon bacillus (and the typhoid bacillus) can precipitate cholesterin and other biliary constituents and hence may take an important part in gall-stone formation. B. coli is also able to produce lesions of the urinary passages, and the majority of all cases of cystitis are to be laid at the door of this organism. According to some writers (Rostoski[2]), as high as 80 per cent. of all cases of urinary tract infection are caused by the colon bacillus. Animal experimentation and the agglutination test support the clinical and pathologic findings in these cases of urinary tract infection. In many cases infection of the bladder appears to take place by way of the urethra rather than through the kidneys from the blood-stream.[3] In some suppurative processes, as in the infection of wounds, B. coli has been recog-

[1] Kramer: Jour. Exper. Med., 1907, 9, p. 319.
[2] Rostoski: Deut. med. Wchnschr., 1898, 24, p. 235.
[3] Bond: Brit. Med. Jour., 1907, 2, p. 1639.

nized as the active agent; and while its share in pyogenic processes is not great, its occasional participation is undoubted.

It is still uncertain to what extent the chronic passage of B. coli from the intestine through the blood into various organs is responsible for certain forms of chronic disease. In the opinion of some investigators this "subinfection" is very important. The conditions under which such penetration of the intestinal wall can take place are not fully known, although there is ground to believe that injuries produced by the hookworm and other parasitic organisms afford an opportunity for invasion of the blood by B. coli. Inflammatory conditions produced by food or wine may likewise favor subinfection. The question of the pathogenicity of B. coli in such circumstances needs further study.

The pathogenic properties of the colon bacillus when confined within the human intestine are not pronounced under ordinary conditions of life. Practically all healthy individuals appear to harbor this organism in their intestinal contents. Excessive sugar fermentation by B. coli with liberation of irritant acids and gas may be responsible for some cases of diarrhea, but there is no evidence that there is any concomitant increase of ability to invade the tissues among the bacilli that take part in this process. Colon bacilli of human origin are practically devoid of power to dissolve and peptonize native proteids such as casein and egg-albumen. It is therefore only in the presence of putrefactive anaërobes or other bacteria capable of peptonizing proteids that colon bacilli aid in excessive intestinal putrefaction.[1]

B. bifidus and B. acidophilus

Morphologically similar to B. coli are the facultative anaërobic bacteria designated as **B. bifidus** (Tissier) and **B. acidophilus** (Moro). Unlike the colon bacillus, however, both these bacilli are gram-positive. According to a number of observers they are the predominant organisms in the digestive tract of healthy human infants. B. bifidus owes its name to a true division or bifurcation of one or both ends of the cell. It is, however, quite polymorphous, and a number of varieties are recognized by students of the intestinal flora.[2] Pure cultures are somewhat difficult to obtain, as

[1] Herter: "The Common Bacterial Infections of the Digestive Tract," New York, 1907, p. 155.

[2] Herter: "The Common Bacterial Infections of the Digestive Tract," pp. 41–45.

B. coli and other associated organisms are likely to overgrow the slow-developing B. bifidus. B. acidophilus is closely related to B. bifidus, but develops readily on a strongly acid medium. The systematic position of these micro-organisms is uncertain, and they are mentioned in connection with the colon bacillus simply for convenience. The generic name Lactobacillus has been used for the group of organisms comprising B. acidophilus, the Bulgarian bacillus, and other bacilli that form lactic acid abundantly from carbohydrates.

THE CAPSULATED BACILLI

A large group of bacilli which are non-motile and heavily capsulated, but which in other respects closely resemble the colon bacillus, are often considered as a separate class of bacteria; but for various

Fig. 65.—Friedländer's pneumobacillus showing the capsules. Blood-agar culture. Fixed with methyl alcohol and stained with carbol gentian violet (Wherry).

reasons they are here included in the same general subdivision with the colon bacillus. Great differences and variations are shown in fermentive power by the different members of this group; several observers have subdivided them on the basis of this characteristic.[1] Pathogenicity is likewise a decidedly variable quantity.

The organism often found in sour milk, and known as **Bacillus [lactis] aërogenes,** bears many points of resemblance to B. coli, and is often found associated with it in the intestine and elsewhere. In general, the fermentive power of B. aerogenes is somewhat greater than that of B. coli. Milk is usually curdled more rapidly, and gas

[1] See, for example, Perkins: Jour. Infect. Dis., 1904, 1, p. 241.

is formed from potato starch. Growth in gelatin is more luxuriant; in gelatin tubes a projecting "nail-head" growth is characteristically produced. Indol is usually produced in peptone solution. Capsule formation occurs in milk cultures.

The most familiar of the pathogenic capsulated bacilli is the form known as Friedländer's pneumobacillus or **Bacillus pneumoniæ** (Fig. 65). In the early studies upon the bacteriology of pneumonia this organism was considered to bear an important relation to the disease, but further investigation has tended to show that, although frequently present in pneumonia as a secondary invader, it is very

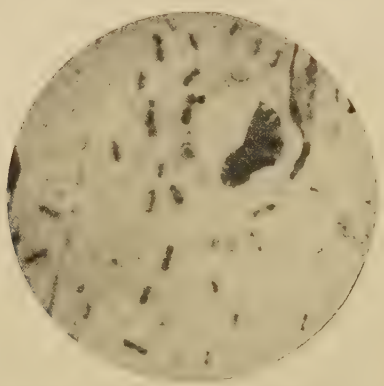


Fig. 66.—Bacillus ozenæ in nasal secretion; methylene-azure; Zettnow prep. (Kolle and Wassermann).

rarely the sole and primary cause. In general morphology and in capsular formation it sometimes resembles the pneumococcus, but is easily distinguished from it by its ready growth upon the ordinary artificial media and by the fact that it loses the stain when treated by Gram's method. The pneumobacillus has been found in suppurative processes in various parts of the body and in some few cases of generalized infection. It is pathogenic for mice and guinea-pigs. The bacillus known as **B. [mucosus] capsulatus** is very similar to, and in the opinion of some observers identical with, Friedländer's bacillus. Some writers prefer to designate the whole group as the "*Mucosus Capsulatus* group."

Organisms of this group have been found in a variety of pathologic conditions. Howard[1] and others have reported cases of hemorrhagic septicemia in a man due to capsulated bacilli. A bacillus

[1] Howard: Jour. Exper. Med., 1899, 4. p. 149.

found in a catarrhal condition of the nose known as ozena (**B. ozenæ,** Abel[1]) belongs to the group of capsulated bacilli (Fig. 66), and a similar capsulated organism has been found in a not very common disease of the upper respiratory tract termed rhinoscleroma. The lesions in rhinoscleroma are of the infectious granuloma type, and a rather characteristic bacillus has been described as occurring quite uniformly in a state of purity deep within the tissue lesions. The "bacillus of rhinoscleroma" is unable, as a rule, to produce gas in any carbohydrate media, and is only feebly pathogenic for the laboratory animals. Some investigators look upon the bacilli found in ozena and rhinoscleroma as secondary invaders and as identical with Friedländer's pneumobacillus. Though this is an extreme view, the etiologic relation of these organisms to the affections with which they are associated is not certainly established. By use of the precipitin method, however, von Eisler and Porges[2] have succeeded in differentiating the bacilli of ozena and rhinoscleroma from the pneumobacillus.

[1] Abel: Ztschr. f. Hyg., 1896, 21, p. 89.

[2] Von Eisler and Porges: Centralbl. f. Bakt., Orig., 1906, 42, p. 660.

CHAPTER XVII

THE GROUP OF COLON-TYPHOID BACILLI (Continued)

Subdivision 2

The Paratyphoid-Enteritidis Group[1]

Bacillus enteritidis.—Under this head may be included certain bacilli isolated from various meat-poisoning epidemics on the continent of Europe and in England. The organism known in laboratories as *Gärtner's bacillus* is typical of the class. It was found by Gärtner (1888)[2] under the following circumstances: The flesh of a diseased cow which was sold for food in a village in Saxony was partaken of by a number of persons, fifty-seven of whom became ill. One young man consumed 800 grams of raw meat and died in about thirty-five hours. From the organs of this fatal case Gärtner isolated a micro-organism which he called B. enteritidis (Fig. 67); the same organism was obtained also from the flesh of the diseased cow. Similar bacteria have been encountered in a case of meat-poisoning in Brussels and in other outbreaks in Germany and England. Outbreaks of disease among wild rats and mice and also among laboratory rodents have been found due to this species. The Danysz virus, sometimes used for exterminating rats and mice, is probably a strain of B. enteritidis. The biologic characters of B. enteritidis and its varieties correspond very closely with those of the so-called hog-cholera bacillus.

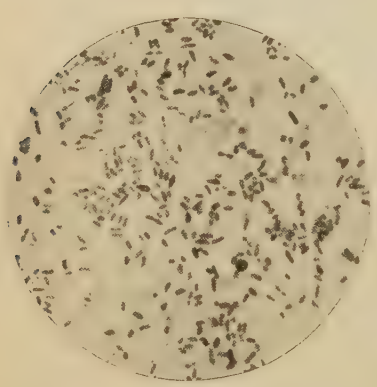


Fig. 67.—Bacillus enteritidis, Gärtner; pure culture; van Ermengem prep. (Kolle and Wassermann).

[1] Sometimes designated as the genus *Salmonella.*

[2] Gärtner: Korresp. d. allg. ärztl. Vereins. von Thüringen, 1888, 17, p. 233.

Bacillus Suipestifer.—A bacillus associated with certain diseased conditions of swine, but probably in most cases a secondary invader, occupies an independent position in this group culturally and serologically (Table, p. 307). It has been isolated in a few human "food-poisoning" outbreaks as, for example, as in one due to tapioca pudding.[1]

The bacillus associated with hog-cholera was discovered in 1885 by Salmon and Smith.[2] Some confusion in nomenclature at first arose from the fact that the organism now known as B. suipestifer was unfortunately first described as "the bacterium of swine plague." The name of swine plague has been since limited to another disease of swine, differing from hog-cholera both in its morbid anatomy and as regards the biologic characters of the specific organism associated with it. Owing to the similarity between the symptoms of hog-cholera and those of swine plague (*Schweineseuche*), the differential diagnosis of these two diseases can often be made only upon the basis of the postmortem and bacteriologic findings. The more important differences between the two species of microbes are shown by the following comparison:

1. **B. suipestifer.**—Actively motile, with from three to nine flagella. Vigorous growth in nutrient broth and moderate growth on potato. Renders milk at first slightly acid, then strongly alkaline, and dissolves the casein. Ferments dextrose with gas-production. Pigs that have fasted for twenty-four hours, when fed with cultures, develop extensive intestinal lesions which result fatally.

2. **B. suisepticus.**—Belongs to the hemorrhagic septicemia group. Nonmotile. No flagella demonstrated. Growth in nutrient broth moderate or feeble. No growth on potato. Acid is generally produced in milk, but not in sufficient quantity to cause coagulation. Acid is produced in dextrose broth, but no gas. Cultures fed to pigs usually produce no effect.

True hog-cholera is widely disseminated throughout the central United States, and also prevails in Great Britain and on the continent of Europe. An acute and a chronic form have been reported, the latter being apparently the more common. Although the micro-organism known as Bacillus suipestifer is found in some epidemics of hog-cholera, doubt has been thrown upon its causal relation to the disease by the fact that it is not found in all cases, and especially by the discovery that the disease can be transmitted to healthy animals by inoculation with diseased body-fluids filtered through the finest porcelain filters.[3] Since the disease can be caused by a filterable virus, the so-called hog-cholera bacillus must be considered to have a very subordinate rôle, if any, in this infection.[4] The relation between hog-cholera and swine-plague is not yet fully cleared up. Some investigators maintain that both diseases are due to one and the same filterable virus and that the differences are due altogether to the associated bacilli, B. suipestifer or B. suisepticus.

[1] Krumwiede, Provost, and Cooper: Jour. Med. Res., 1922, 43, p. 53.

[2] Smith: Centralbl. f. Bakt., 1891, 9, p. 253.

[3] DeSchweinitz and Dorset: Bureau of Animal Industry, 1903, Circular 41; Boxmeyer, Jour. Infect. Dis., 1905, 2, p. 359.

[4] See especially Dorset, Bolton, and McBryde: "The Etiology of Hog-cholera," Bureau of Animal Industry, Bull. No. 72, 1905.

Paratyphoid Bacilli. –Achard and Bensaude[1] were the first to isolate an atypical organism from human tissues during convalescence from a typhoid-like disease. Later Gwyn[2] reported a case which apparently presented all the clinical symptoms of typhoid fever, but in which the serum reaction for the typhoid bacillus was lacking. From the blood Gwyn isolated a bacillus closely akin to B. suipestifer and B. enteritidis, which agglutinated with the patient's serum. Since that time a number of investigators[3] in different parts of the world have isolated similar organisms from the blood of patients suffering from a disease that, so far as clinical symptoms are concerned, is sometimes substantially identical with typhoid fever. Many cases of "paracolon" or "paratyphoid" infection show a tendency to run a rather mild course and are marked by a sudden onset with chills, but are otherwise very similar to infections with the true typhoid bacillus. A certain proportion of the negative results reported with the agglutination test in apparent typhoid fever can be plausibly accounted for by the supposition that a paratyphoid rather than a typhoid bacillus was the exciting cause of the attack. Many isolated cases of paratyphoid infection have been observed, and one small epidemic due to water infection has been attributed to an organism of this group.[4] It is not yet known what proportion of cases clinically resembling typhoid fever are due to paratyphoid bacilli. In a series of thirty cases studied by Ruediger[5] in Chicago, paratyphoid organisms were isolated from the blood in two cases, typhoid bacilli in seventeen cases. Some cases of paratyphoid infection are accompanied with severe diarrhea and resemble the so-called cholera nostras. The observations of Hetsch[6] indicate that they might be mistaken clinically for Asiatic cholera.

The pathologic anatomy of paratyphoid fever is not very fully known, since, owing to the relatively low mortality, few thorough autopsies have been made. Up to the present the findings indicate that the lymphatic system is not as generally involved as in typhoid

[1] Achard and Bensaude: Soc. méd. des Hôp. de Paris, 1896, 3d S., 13, p. 679.
[2] Gwyn: Bull. Johns Hopkins Hosp., 1898, 9, p. 54.
[3] Schottmüller: Ztschr. f. Hyg., 1901, 36, p. 368; Buxton: Jour. Med. Res 1902, 8, p. 201.
[4] See Jürgens: Ztschr. f. Hyg., 1903, 43, p. 372.
[5] Ruediger: Trans. Chicago Path. Soc., 1903, 5, p. 187.
[6] Hetsch: Klin. Jahrb., 1907, 16, p. 267.

fever; this is especially evidenced by the absence of the characteristic typhoid ulcerations of the Peyer's patches. Broadly speaking, the lesions of paratyphoid infection are those of a severe gastro-enteritis.

Two varieties of paratyphoid bacilli are recognized, usually designated as type **A** and type **B**. Type **B** is probably the more widely distributed, and is found in by far the greater number of cases of paratyphoid infection. Most cultures of type **A** produce alkali in litmus milk much more slowly (rarely in less than fourteen days) than type **B** cultures (usually in four to five days). The **A** strains do not ferment xylose; they attack dulcit slowly. The **B** strains ferment xylose and attack dulcit rapidly.[1] Most strains of Bacillus paratyphosus **B** blacken lead acetate agar in eighteen to twenty-four hours, while typical B. paratyphosus **A** strains fail to do this.[2] The specific agglutinins and other antibodies produced by the two types of paratyphoid bacilli are different. The cases of illness in which type **A** is found resemble typhoid fever. The pathogenicity of type **A** for the lower animals is less than that of type **B** and corresponds more nearly to that of the typhoid bacillus.

Bacilli of the paratyphosus **B** type appear to be serologically of two groups, one (var. Schottmüller) found chiefly in cases of human paratyphoid fever and sometimes in diseased swine, the other (var. Aertrycke) found in food-poisoning outbreaks in man and in various rodent infections.[3] The latter seem identical with what English writers have called the "mutton" type.

Epidemiologically paratyphoid infection appears to be frequently due to food poisoning. Milk and meat probably play quite a part in infection, and water is sometimes implicated. The fact that both typhoid and paratyphoid fever may be caused by polluted water, and that cases may exist side by side in the same epidemic undifferentiated, suggests that the full extent of water-infection may not now be recognized.

Organisms superficially resembling paratyphoid bacilli are widely distributed in nature. They are found not infrequently in the healthy human intestine, and occur often also in the intestine of swine, mice, and other animals. It is improbable that such

[1] Jordan: Jour. Infect. Dis., 1917, 20, p. 457.
[2] Jordan and Victorson: Jour. Infect. Dis., 1917, 21, p. 571.
[3] Jordan: Jour. Infect. Dis., 1923, 33, p. 567.

bacilli can be definitely identified either as B. paratyphosus or B. suipestifer. Certain English bacteriologists look upon these bacteria from normal animals not as genuine paratyphoid bacilli, but as saprophytic "para-Gärtner" forms. Agglutination experiments made by the absorption method show a difference between these bacilli and cultures of B. paratyphosus and B. enteritidis from human infections.

No connection of human paratyphoid fever with specific diseases of the lower animals has been certainly established. In spite of the extraordinarily close resemblance between B. paratyphosus, B. suipestifer, B. psittacosis, and the bacillus of mouse typhoid (B. typhi murium), extending even to agglutinating and immunizing tests, a difference in pathogenicity seems to exist, and reciprocal infections of the domestic animals and man are, to say the least, very rare. Shibayama,[1] however, has reported a number of instances in which cakes containing mouse-typhoid bacilli (probably B. enteritidis) were eaten by mistake and severe gastro-enteritis ensued.

The existence of a violent gastro-intestinal form of paratyphoid fever and a milder typhoid-like form, especially in the meat-poisoning cases, may possibly be explained by supposing that a simple infection occurs in the latter case, while in the more severe and sudden form intoxication from the ingestion of formed poison is mainly responsible. The resistance of the paratyphoid poison to heat is a fact in favor of this explanation.

A number of other organisms of more or less bacteriologic importance belong to the paratyphoid-enteritidis group. Infectious abortion of mares is due to a definitely characterized member of the group, known as **B. abortivo-equinus.**[2] **B. psittacosis** was found in a highly fatal, pneumonia-like disease of human beings which broke out in Paris in 1892, causing forty-nine cases and sixteen deaths. It was shown that sick parrots from South America were the starting-point of this outbreak. Other cases of this peculiar infection, in some instances likewise communicated by parrots, have been recorded by Leichtenstern,[3] in Cologne, and by other

[1] Shibayama: Münch. med. Wchnschr., 1907, 54, p. 979.

[2] See *e. g.*, Good and Smith: Bull. 204, Ky. Agr. Expt. Station, 1916; Murray: Jour. Infect. Dis., 1919, 25, p. 341.

[3] Leichtenstern: Centralbl. f. allg. Gesundh., 1899, 18, p. 241.

observers.[1] There is a good deal of doubt, however, whether B. psittacosis is the real cause of the pneumonia transmitted to man by parrots. Some investigators incriminate a special variety of streptococcus.[2]

An organism isolated by Danysz[3] from an epidemic among harvest mice belongs to this group. Under the name of the **Danysz virus** it has been widely used in attempts to exterminate mice and rats. The virus does not seem to be readily transmitted from rat to rat under natural conditions, and its practical value is not so great as often claimed. Its use cannot be said to be altogether without danger to man.

Much interest attaches to the interrelationship of the bacilli composing the paratyphoid group, and elaborate attempts have been made to utilize the agglutination test in disentangling the biologic affinities.[4] A sharp distinction may be made on the basis of agglutination reactions between organisms culturally similar. At least five groups become in this way recognizable: Bacillus enteritidis (Gärtner's bacillus); B. paratyphosus **A;** B. paratyphosus **B,** Type Schottmüller and Type Aertrycke; B. abortivo-equinus; and B. suipestifer. Four of these have been reported as found in outbreaks of human food poisoning.[5]

[1] Vickery: Med. News, 1904, 85, p. 780.

[2] Bachem, Selter, and Finkler: Klin. Jahrb., 1910, 23, p. 539.

[3] Danysz: Ann. de l'Inst. Past., 1900, 14, p. 193.

[4] Krumwiede: Jour. Med. Res., 1916, 29, p. 355; 30, pp. 55 and 357; 1917, 31, p. 509. Jordan: Jour. Infect. Dis., 1917, 20, p. 457; 21, p. 554; 1918, 22, p. 252.

[5] Jordan: "Food Poisoning," The University of Chicago Press, 1917, p. 58.

CHAPTER XVIII

THE GROUP OF COLON-TYPHOID BACILLI (Continued)

Subdivision 3

The Typhoid-Dysentery Group

THE TYPHOID BACILLUS (B. TYPHOSUS)

The typhoid bacillus was discovered by Eberth in 1880[1] in the mesenteric glands and the spleen of persons dying from typhoid fever. With the microscope the bacilli are usually seen in stained sections of the spleen or liver, where they occur in groups or masses rather sharply focalized, scattered individuals not being often found. In 1884 Gaffky[2] succeeded in growing Eberth's bacillus upon culture-media, and since that time evidence has slowly accumulated that this organism is the cause of typhoid fever.

At first difficulties stood in the way of the general acceptance of this view. Eberth's bacillus proved pathogenic for the lower animals when injected intraperitoneally or intravenously; but it was not possible to produce typhoid fever in animals by feeding them with small numbers of bacilli, the natural mode of infection in man, nor was it possible by any mode of infection to reproduce the gross lesions of human typhoid fever. Strong evidence of causal relationship, on the other hand, was brought out in the discovery of "Pfeiffer's phenomenon" (p. 161) and in the "Gruber-Widal test" or agglutination reaction. Thoroughly characteristic typhoidal lesions are said to have been produced in the chimpanzee by feeding with typhoid bacilli,[3] and pure cultures of the Eberth-Gaffky bacillus swallowed with suicidal intent have given rise to typhoid fever in man (Duflocq and Voisin[4]).

Characteristics of the Typhoid Bacillus.—The typhoid bacillus is a short, plump rod, its dimensions ranging, as a rule, from 1 μ to 3 μ in length and from 0.5 μ to 0.8 μ in breadth (Figs. 68 and 69).

[1] Eberth: Archiv. f. path. Anat., 1881, 83, p. 486.
[2] Gaffky: Mitt. a. d. k. Gesund., 1884, 2, p. 372.
[3] Grünbaum: Brit. Med. Jour., 1904, 1, p. 817.
[4] Duflocq and Voisin: Arch. gén. de méd., 1903, 2, p. 2197.

It is actively motile and possesses more flagella than B. coli (Fig. 70). The cell inclusions at one time mistaken for spores by Gaffky and others were probably either vacuoles or metachromatic granules. As regards growth upon media, it may be said that, as a rule, the typhoid bacillus is able to grow far less luxuriantly than the colon bacillus, and the chemical changes that it is able to effect are much less numerous and profound than those that are brought about by the latter organism. Consequently, the cultural characteristics of the typhoid bacillus are distinctly negative as compared with the positive character of other members of the group; in peptone solution no indol is produced; in dextrose broth and agar, acid is produced but no gas is formed (Fig. 71); no acid is produced from lactose and saccharose; milk is not curdled, and although some strains produce a small amount of alkali in milk and in litmus whey, the change in reaction is seldom, if ever, as great as that produced by most of the members of the B. enteritidis group. The colonies upon gelatin are thin, bluish-white expansions with irregularly notched margins, and are, as a rule, not so large or thick as the colonies of the colon bacillus (Figs. 72 and 73). Typical cultures of the typhoid bacillus grow upon the surface of acid potato, but the growth is thin, moist and colorless, and forms the so-called

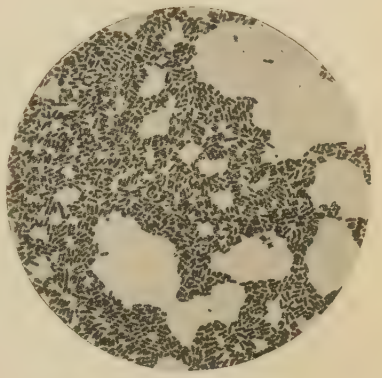


Fig. 68.—Bacillus typhosus, twenty-four-hour agar culture; × 650 (Heim).

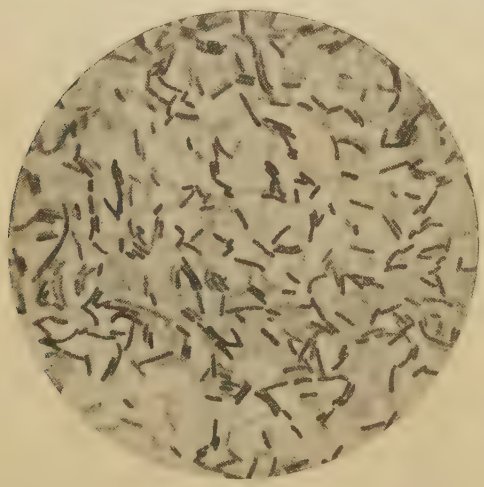


Fig. 69.—Bacillus typhosus. Impression preparation from gelatin plate. Fuchsin; × 1000 (Hicks).

invisible film, which is strikingly unlike the profuse brownish growth of the typical colon bacillus. On pieces of potato with an alkaline reaction, the growth is more like that of the colon bacillus. No final diagnostic value at present attaches to the growth upon potato, owing to the wide variations both in the reaction of potatoes and in the behavior of different strains of bacilli, but nevertheless the growth on potato is often of determinative importance. On acid potato typical cultures of typhoid bacillus and colon bacillus can be readily distinguished. A similar distinction appears upon Heineman's substitute for potato.[1]

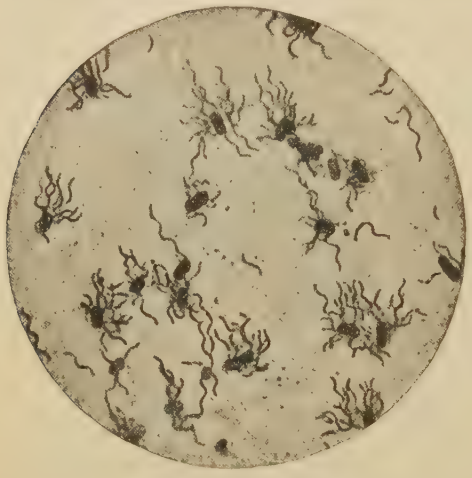


Fig. 70.—Bacillus typhosus, from an agar culture six hours old, showing the flagella stained by Löffler's method; × 1000 (Fränkel and Pfeiffer).

The more salient points of difference between the typhoid bacillus and the other organisms of the group have been already presented in tabular form (p. 307). Since the various bacilli belonging to the colon-typhoid group are in many respects similar, and are frequently found side by side in infected organs, in polluted water, and elsewhere, the application of elaborate and extended comparative tests is requisite for a sure diagnosis. Much ingenuity has been expended in devising new methods for isolation and identification, but it is now generally admitted that any satisfactory identification of the typhoid bacillus through

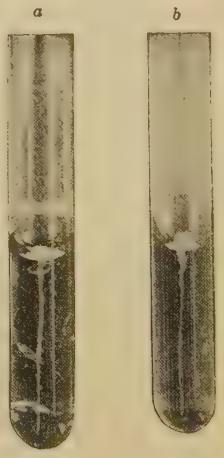


Fig. 71.—*a*, Bacillus coli in dextrose agar, showing gas bubbles; *b*, Bacillus typhosus.

[1] Heineman: Jour. Infect. Dis., 1907, 4, p. 282.

a single test is impossible, and that only a comprehensive study of a considerable number of biologic attributes enables an identification to be made with a reasonable degree of certainty. The advance of bacteriologic investigation in this field has been accompanied by a steady increase in the number of tests that must be applied in order to arrive at a satisfactory identification of the specific organism of typhoid fever.

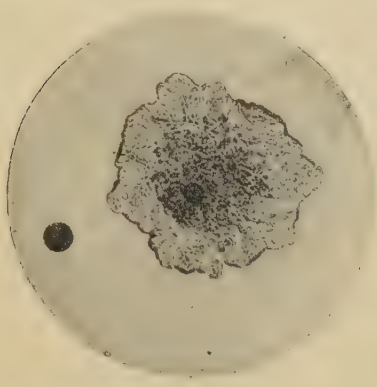


Fig. 72.

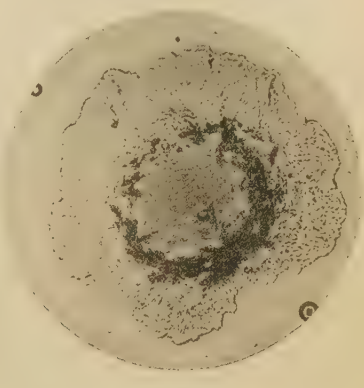


Fig. 73.

Figs. 72 and 73.—Gelatin colonies two days old of Bacillus typhosus (Fig. 72) and Bacillus coli (Fig. 73); × 21 (Heim).

Methods for Isolating the Typhoid Bacillus.—A large number of methods have been proposed to facilitate the speedy isolation of the typhoid bacillus from polluted water, fecal discharges, and other suspected sources. One of the chief difficulties that these methods seek to overcome is the separation of the typhoid bacillus from the colon bacillus and allied varieties. The problem consequently resolves itself into the discovery of a medium which shall both favor the development of the typhoid bacillus, and also assist in differentiation between B. typhosus and B. coli. It has proved a relatively simple matter to suppress the common saprophytic bacteria of water, soil, and sewage by the use of high temperatures, antiseptics, and other inhibitive influences to which the members of the colon-typhoid group are especially resistant, but the elimination of the always abundant B. coli cannot be so readily accomplished. The colon bacillus is in general more resistant than the typhoid bacillus and is far more richly endowed with the ability to initiate various reduction and fermentation processes.

The active motility of the typhoid bacillus as compared with the sluggishness of nearly all strains of colon bacilli is one of the few positive characteristics of diagnostic value. Several of the methods recommended for the isolation of the typhoid bacillus are based upon this quality. These methods have frequently given excellent results in the hands of the inventor, but have not always proved equally available in the hands of other experimenters.

Several varieties of colored media, some of which contain inhibitory agents, have won favor in recent years. The medium of Drigalski and Conradi[1] has been employed by many investigators. Various modifications of the original medium have been introduced. One formula successfully used by Harris is the following:

Dextrose-free broth	2000 c.c.
Nutrose	10 grams.
Agar	40 grams.

Boil, dissolve, neutralize to phenolphthalein, autoclave at 120° for five minutes. Clarify with whites of four eggs and filter. Then add: lactose, 30 grams; 1 per cent. litmus solution, 260 c.c.; crystal violet, 20 c.c. of 0.1 per cent. aqueous solution. The crystal violet exercises a marked restraining influence, most of the purely saprophytic bacteria never coming to development. In fourteen to twenty-four hours at 37° C. the colonies of B. coli appear red, opaque, and rather large, while the colonies of B. typhosus are relatively small and transparent and blue. Not all of the translucent blue colonies are typhoid bacilli, however, and further tests are necessary to establish identification. Plates made with the Drigalski-Conradi medium have facilitated greatly the isolation of typhoid bacilli from excreta.

Endo[2] has prepared a fuchsin-lactose-agar decolorized by sodium sulfite which makes possible a somewhat similar differentiation between B. coli and B. typhosus, and is now widely used. The formula is as follows:

Three per cent. nutrient agar	1000 c.c.
Lactose	10 grams
Filtered saturated solution of basic fuchsin in 95 per cent. alcohol	2 c.c.
Sodium sulfite solution (10 per cent. of the dry powder)	25 to 30 c.c.

[1] Drigalski and Conradi: Ztschr. f. Hyg., 1902, 39, p. 283.
[2] Endo: Centralbl. f. Bakt., 1904, I, Orig., 35, p. 109.

The Arnold steam-bath is to be used for sterilization. The medium does not keep in good condition for more than about two weeks, consequently the fuchsin and sodium sulfite should not be added to the lactose agar until shortly before the medium is to be used. The reaction is important. The best results are obtained where the medium is slightly (0.1 to 0.2 per cent.) acid to phenolphthalein (P_H 7.8–8.2). After eighteen to twenty-four hours' incubation the typhoid colonies appear as clear, colorless, glistening drops on the background of the uncolored medium. The colonies of B. coli are red. Equally good results are obtained with the eosin methylene-blue medium as modified by Levine.[1] On this medium the colonies of B. coli are a deep purple, while typhoid colonies appear almost colorless.

Suspicious colonies on the Endo medium may be advantageously transferred to tubes of Russell's medium.[2] This consists of 5 per cent. aqueous solution of litmus (3 to 5 per cent.) added to plain agar (2 or 3 per cent.) with a P_H of about 7.6. One per cent. of lactose and $\frac{1}{10}$ of 1 per cent. dextrose are finally added to the medium, which is then tubed and sterilized. On this double sugar medium the typhoid bacillus gives a characteristic appearance, the upper part of the tube being uniform in color, while the lower part is a brilliant red. B. coli shows abundant gas and acid formation throughout the medium. The paratyphoid bacilli produce a small amount of gas in the lower half of the tube, while the upper part of the medium is, as a rule, unchanged. B. fecalis leaves the medium unaltered or slightly bluer.

Especially favorable results have been obtained by Havens and his associates[3] with a medium containing brilliant green dye. The high proportion of positive findings secured by their method warrants a full description of the technique. Since the market brands of brilliant green dye lack uniformity a stock solution is made of a 5 per cent. solution in N/1 HCl, evaporated to about $\frac{1}{20}$ of its volume over a water bath and diluted with distilled water to make a 5 per cent. solution. This stock solution is then added to a series of wide-mouthed bottles each containing 15 c.c. of bile

[1] Levine: Iowa State College of Agriculture and Mechanical Arts, Bull. 62, 1921, p. 117.

[2] Russell: Jour. Med. Res., 1911, 25, p. 217.

[3] Havens and Dehler: Jour. Preventive Med., 1927, 1, p. 359.

(dehydrated—Difco) in sufficient quantities to make a series of dilutions from 1 : 100 to 1 : 500, or higher, with 20 to 25 per cent. intervals between the dilutions. The bottles are corked, and sterilized in the autoclave. The most suitable concentration of the dye can then be determined by adding to each bottle of the series about half a gram of feces and a moderate inoculation of typhoid bacilli. A properly prepared medium inhibits the normal fecal bacteria and allows B. typhosus and allied forms to multiply. The inoculated bottles are allowed to stand at room temperature for 24 hours, and plates are then streaked with a loopful from the surface of each bottle without agitation of the contents. Plating should be repeated on the two succeeding days. The results, as indicated by a high proportion of typhoid colonies and a low proportion of B. coli, determine the most advantageous concentration of the dye. A working quantity of the brilliant green bile medium may then be prepared and stored at a low temperature. Preliminary incubation in this medium is followed by plating. Havens regards brilliant green Endo medium as satisfactory. The proper concentration of the brilliant green (usually between 1 : 250,000 and 1 : 500,000) must be determined for each lot of agar prepared. Suspected colonies may be transferred to the Russell double sugar tubes, and those cultures that react positively subjected to complete identification tests including agglutination.

Distribution of the Typhoid Bacillus in Nature.—The typhoid bacillus is by preference a parasite. Outside the human body it has been found only in those situations where it could be more or less directly traced to an origin in the discharges of a typhoid patient or convalescent. Many of the earlier reported findings of this organism in water and soil cannot now be given credence, owing to the inadequacy of the identification tests to which the cultures were subjected. Up to the present time relatively few well-authenticated instances have been recorded in which the typhoid bacillus has been found in water, soil, and similar situations. Laboratory experiments have shown that the typhoid bacillus can survive in sterile water in glass vessels for upward of three months, and for possibly two or three weeks in unsterilized ground or surface water. Other evidence indicates that the bacillus is able to travel in water a distance of at least 140 kms. (Gärtner[1]) and to retain its vitality

[1] Gärtner: Klin. Jahrb., 1902, 9, p. 335.

in natural bodies of water for at least four or five days (Jordan, Russell, and Zeit[1]). It is possible that water may continue to be the vehicle of infection during a much longer period, but the available data point to a comparatively short duration of life of the specific germ in the water of flowing streams (Jordan, Russell, and Zeit). Under ordinary conditions no multiplication of the typhoid bacillus takes place in water, even when a considerable amount of organic matter is present, but, on the contrary, a steady decline in numbers goes on. The history of typhoid epidemics tends to show that sewage pollution is to be feared chiefly when the sewage is fresh, and that the danger of infection diminishes progressively with the lapse of time.

In soil and in the fecal matter of privy vaults the duration of life of the typhoid bacillus is much longer than in water. In some observations on the stored feces of typhoid patients and typhoid carriers, typhoid bacilli have been found alive for as long as 52 days.[2] The evidence that any genuine multiplication can take place in the soil is not convincing, but it has been proved that the bacillus may be carried by water-currents to a considerable distance from the point where it was first introduced. Infection of wells and small water-courses is thus brought about sometimes by the washing of bacilli out of soil in which they may have lain dormant for many weeks. The persistence of typhoid fever around certain habitations may be plausibly explained on the supposition of an extensive soil contamination. There is no doubt that the practice of using human excrement for manuring vegetable gardens entails a danger no less real because often unrecognized.

The history of typhoid epidemics indicates that air-borne infection is, to say the least, exceedingly rare. Sewer air, so far as known, is never the vehicle by which the specific germ of typhoid fever is conveyed from one place to another.

Pathogenicity for the Lower Animals.—It has long been known that house pets and domestic animals do not become affected during epidemics of typhoid fever. Attempts to reproduce typical typhoid fever in the animals ordinarily used for laboratory experiments have not met with much success. The early experiments

[1] Jordan, Russell, and Zeit: Jour. Infect. Dis., 1904, 1, p. 641.
[2] Jordan: Jour. Infect. Dis., 1926, 38, p. 306.

of feeding rabbits, guinea-pigs, and mice with typhoid cultures or infected food were negative.

Later observers have obtained a more positive result. Remlinger,[1] for example, succeeded in producing a genuine infection by feeding vegetables smeared with typhoid bacilli to fasting rabbits and rats. But even in these experiments it must be admitted that there is no precise reproduction of the ordinary clinical picture of human typhoid fever. Grünbaum,[2] however, has reported interesting results from feeding chimpanzees with typhoid cultures in milk and broth. On killing the animals twelve days after infection characteristic typhoid lesions were found in the ileum, and the typhoid bacillus was recovered from the spleen.

As regards the ordinary domestic animals, the available data point to a rapid destruction of the specific bacilli when they are introduced into the alimentary tract. It has been shown[3] that when the bacillus is fed to animals in considerable quantities it does not reappear in the feces; hence the fear that cattle drinking polluted water might become a means of spreading the disease through the multiplication of typhoid bacilli in their intestines, even though the animals themselves might not suffer from the disease, does not seem justified.

Intraperitoneal injection of typhoid bacilli has much the same effect upon animals as injection with colon bacilli (Pfeiffer and Kolle[4]). When introduced into the peritoneum in considerable quantity($\frac{1}{50}$ to $\frac{1}{30}$ of a loop of a young, virulent agar culture), many strains of typhoid bacilli evince pathogenic properties. General symptoms of a non-specific character are set up and result fatally (six to eight hours) when a susceptible animal is inoculated with a considerable number of bacilli of a sufficiently virulent strain. Although a genuine but slight multiplication of bacilli takes place in the peritoneum and attests the occurrence of a true infection, neither the symptoms nor lesions of this intraperitoneal typhoid bear any close resemblance to the typhoidal processes in man. The substance that is toxic for the animal organism is contained in the bodies of the bacilli, and is not a secretion product, as is shown by experiments with young sterilized cultures.

[1] Remlinger: Ann. de l'Inst. Past., 1897, 11, p. 829.
[2] Grünbaum: Brit. Med. Jour., 1904, 1, p. 817.
[3] Stokes: Maryland Med. Jour., Nov., 1900.
[4] Pfeiffer and Kolle: Ztschr. f. Hyg., 1896, 21, p. 203.

Pathogenicity for Man.—Typhoid fever (Eng., *enteric fever;* Ger., *Abdominaltyphus* or *Typhus;* Fr., *la fièvre typhoide*) was for long one of the most widespread and important of all bacterial diseases. In the United States in the census year, 1900, there were reported 35,379 deaths from this disease, and this number was probably considerably below the true figures. The number of deaths reported indicates that there were at that period at least 350,000 cases of typhoid fever in a single year in a population of about 76,000,000, and that in the course of a decade perhaps one person in every twenty or twenty-five contracted typhoid fever. Typhoid infection is caused by taking into the mouth germs discharged in human urine or feces. The conditions that make this possible do not imply that a very advanced stage of civilization has been reached. While the prevalence of typhoid has greatly diminished in recent years throughout a large part of the world there were nevertheless in 1925 in the United States 8287 deaths from typhoid in a population of approximately 103,000,000. The disease has become relatively very rare in most modern European[1] and American cities.[2] This improvement is largely due to the chlorination of public water supplies and the pasteurization of milk.

The physiologic accompaniments of typhoid fever are many and variable, so that a diagnosis by clinical methods is often difficult, especially in the early stages of some cases. The common symptoms comprise frontal headache, want of appetite, nose-bleed, the development of rose spots on the abdomen, muscular weakness and diarrhea. There is, as a rule, a general step-like rise of temperature during the first week or ten days. On autopsy the intestinal walls are usually found extensively ulcerated, Peyer's patches and the solitary glands of the intestine being particularly involved and containing the specific bacillus. Perforation of the intestinal wall as a consequence of ulceration is a serious and not infrequent occurrence. The spleen is enlarged and congested, and usually contains large numbers of typhoid bacilli.

In addition to the more or less constant symptom-complex, recognized as the definite disease of typhoid fever, there are certain other pathologic conditions of the human body with which the typhoid bacillus stands in causal relation. Inflammation of the

[1] Jour. Amer. Med. Assoc., 1925, 85, p. 1890.
[2] Jour. Amer. Med. Assoc., 1927, 88, p. 1148.

urinary bladder (cystitis) sometimes occurs. The gall-bladder also is very commonly affected and severe inflammations of this organ are sometimes noted.

Suppurative and inflammatory processes (metastases) may be kindled by the typhoid bacillus in many parts of the body. The osseous system seems especially open to attack, and affections of the periosteum, the bone-marrow and the joints have been traced to infection with B. typhosus. Osteomyelitis may develop as long as six or seven years after recovery from typhoid fever. Particular interest attaches to these cases, since they show that the typhoid bacillus can remain for years in contact with the human tissues and presumably be exposed to the action of the protective substances in the blood without losing its virulence.

Other parts and organs of the body are more rarely invaded by the typhoid bacillus, but under certain conditions almost any organ may be attacked. The presence of the bacillus has been reported in a brain abscess.[1] The cerebral and meningeal symptoms occurring in many cases of typhoid fever are directly connected with the localization of the typhoid bacilli in the meninges, and the bacilli have been obtained in the fluid drawn by lumbar puncture.[2]

Secondary or mixed infections, especially with the pyogenic cocci and the pneumococcus, are not at all uncommon, and sometimes cause serious complications. Mixed infections with the tubercle bacillus, the diphtheria bacillus, and B. anthracis (Karlinski[3]) have been known to occur.

The intestine has long been considered as the main if not the sole portal of entry of the typhoid bacillus, but other possibilities have been suggested by recent investigation. The actual evidence in favor of invasion of the body through the tonsils and gastric mucosa is considered by some investigators to be quite as strong as the evidence for intestinal penetration. In any case there is no doubt that typhoid fever is a general and not a localized infection.

Distribution of Bacilli within the Body of the Patient.—In correspondence with the frequency of intestinal symptoms and lesions typhoid bacilli might be expected to be commonly present in the feces and intestinal contents of typhoid patients. Great

[1] McClintock: Amer. Jour. Med. Sci., 1902, 123, p. 595.
[2] Cole: Johns Hopkins Hosp. Rept., 1904, 12, p. 379.
[3] Karlinski: Berl. klin. Wchnschr., 1888, 25, p. 866.

difficulty, however, is often experienced in isolating them from feces, owing partly, no doubt, to their association with a multitude of colon bacilli and related organisms. Many special methods, some of which have been already described, have been devised for facilitating isolation. The largest number of positive findings are reported as made between the seventh and twenty-first days of the disease, so that in the majority of cases a positive result is not obtained until such a period that the nature of the malady is evident on clinical grounds. Part of the difficulty in isolation is due to the real scarcity of typhoid bacilli in feces. The fact that typhoid bacilli usually occur in small numbers in the stools of typhoid patients and are not infrequently altogether absent has led some recent investigators to believe that the bacilli do not multiply in the intestinal contents except under unusual conditions. It is believed also that the typhoid bacilli in the intestine come chiefly from the bile.[1]

In the urine typhoid bacilli are found in about 25 per cent. of all cases, sometimes in conjunction with the colon bacilli, but often in pure culture. They may occur in enormous numbers: in some cases observers have found from 100,000,000 to 500,000,000 in a cubic centimeter of urine. Cystitis is occasionally produced as a result of urine infection, but is not a necessary sequel. The urine may remain infectious far into convalescence unless some method of sterilization of the bladder be resorted to. The administration of urotropin by mouth has sometimes proved useful for this purpose, and good results have been obtained by washing out the bladder with a solution of mercuric chlorid (Richardson[2]). Typhoid bacilli sometimes persist in the urine for weeks and months after recovery (seven years, Young[3]). The danger of dissemination of typhoid fever by means of the infected urine of convalescents is especially great, and appropriate methods for the treatment of the urine as well as the feces of typhoid patients should be uniformly employed in all cases.

The bile becomes frequently infected during an attack of typhoid fever, as shown by post-mortem examinations. In experiments

[1] For a review of this subject with full bibliography see an article by Pratt, Peabody, and Long: Jour. Amer. Med. Assoc., 1907, 49, p. 846.

[2] Richardson: Jour. Exper. Med., 1898, 3, p. 349.

[3] Young: Johns Hopkins Hosp. Rept., 1900, 8, p. 401.

upon rabbits Blachstein[1] found living typhoid bacilli in the gall-bladder, in one case as long as one hundred and twenty-eight days after intravenous injection and after the other organs had become entirely free. If an inflammatory process is set up in the gall-bladder or duct, typhoid bacilli may continue to be discharged into the intestine with the bile for practically indefinite periods.[2] The epidemiologic significance of persistent bile infection has only recently come to light. The so-called chronic bacillus-carriers are, in many cases at least, persons with typhoid bacilli in their gall-bladders. In the German campaign against typhoid fever in the Rhine provinces, it was observed that a large proportion of all persons who suffer from gall-stones are discharging typhoid bacilli in their feces.

Typhoid bacilli have been found occasionally in the *sputum*, although the majority of cases of pneumonia that occur during the course of typhoid fever are apparently caused by the pneumococcus. There is hence a possibility that typhoid fever may be spread by sputum infection, but it seems rather remote.

The *rose spots* that appear on the abdomen in about 80 per cent. of cases of typhoid fever are to be considered as a specific eruption due to the presence of typhoid bacilli. The earlier attempts to isolate the bacilli from the rose spots usually failed because a large amount of blood was drawn and insufficiently diluted with broth, thus allowing opportunity for the action of the germicidal substances in the blood. By proper methods the bacilli can be isolated from the rose spots at an earlier period in the disease than from the feces, and, indeed, earlier than the agglutination reaction makes its appearance.[3]

The examination by suitable methods[4] of the blood of typhoid patients during life has shown that in the great majority of cases bacilli are present in the blood-stream very early in the course of the disease. Diagnosis is often possible by blood examination earlier than by other methods. After death the bacteria are found in practically all the important organs and tissues.

[1] Blachstein: Bull. Johns Hopkins Hosp., 1891, 2, p. 96.

[2] Seven years, Miller: Bull. Johns Hopkins Hosp., 1898, 9, p. 95.

[3] Neufeld: Ztschr. f. Hyg., 1899, 30, p. 498.

[4] A considerable quantity of blood must be mixed with a much larger quantity of broth or fluid agar immediately after it is drawn, to prevent the action of the germicidal substances in the blood.

The general distribution of the typhoid organism throughout the body has caused some modification in our conception of the pathology of typhoid fever. In the light of all the facts this disease must now be looked upon as a general invasion of the body, particularly of the lymphatic system, rather than as a purely localized infection with its seat in the wall of the alimentary tract.[1] Coleman and Buxton[2] have advanced the view that the lymphatic tissues in the intestinal wall are first invaded, and that thence the bacilli spread to the general lymphatic system and the spleen. After considerable multiplication has occurred (incubation period), the bacilli overflow into the blood and bacteriolysis takes place; the endotoxins which are liberated as the result of the destruction of the bacilli produce the symptoms of typhoid fever.

Epidemiology.—The predilection shown by typhoid fever for certain localities and for certain seasons has given rise to many speculations concerning the influence of atmospheric and telluric conditions, and at one time led to the promulgation by Pettenkofer of a theory of the "ripening" of typhoid germs in the soil. The influence of locality in itself, apart from its possible influence upon the character of the water-supply, does not now seem to be important, although a city with a badly infected water-supply, as was for many years Pittsburgh, Pennsylvania, may become the center of a typhoid-ravaged district. This is not because of any peculiarity of soil or climate, but because new foci of infection are continually being kindled and the disease is kept alive by the great abundance of infectious material. Sedgwick has proposed the apt term *prosodemic* to designate the general diffusion of a disease among the people of a community. The seasonal incidence of the disease in early autumn has not been fully explained.

In recent years many of the most obscure and perplexing facts in the distribution of typhoid fever have had light thrown on them by the discovery of the intimate relation borne by typhoid patients and convalescents to the further spread of the infection. In all the epidemiology of this disease the human being that carries the bacillus is the central figure. Typhoid bacilli leave the body, as

[1] The finding of the bacillus in the blood has led some writers to speak of typhoid fever as a "modified septicemia." There is no good evidence, however, that the bacilli are multiplying in the blood, which is strongly bactericidal.

[2] Coleman and Buxton: Jour. Med. Res., 1909, 21, p. 83.

a rule, in either the feces or the urine, pass into the external world, and find their way more or less circuitously to the alimentary tract of another individual. There proliferation may again begin, and a new focus of infection comes into existence. Outside of the human body, multiplication, if it occurs at all, is insignificant, and for practical purposes may be neglected as a factor in the dissemination of the disease. So far from multiplying freely, typhoid bacilli, when discharged from the body, undergo progressive diminution in numbers; it is probable that the majority perish under ordinary conditions within a few days, although in masses of fecal material some bacilli may remain alive for relatively long periods. The principal channels by which the typhoid bacillus makes its way to fresh victims will be briefly considered.

(*a*) *Water.*—There is unfortunately too intimate a connection between sewage-disposal systems and water-supplies. The remarkably rich epidemiologic literature of typhoid fever shows a great preponderance of large epidemics of water-borne infection. Schüder,[1] in a compilation of 638 epidemics of typhoid, found that 71 per cent. were attributed to contaminated drinking-water. Manifold instances have been reported where typhoid fever has been definitely traced to the use of water from a polluted well or spring, only those individuals being affected who used the water from the contaminated source. Weichselbaum[2] records the curious case of a certain house in Stuttgart which was invaded by typhoid fever during an epidemic caused by a town water supply. Only those persons living on the second and fourth floors of this house were affected; the dwellers on the first and third floors were exempt and were found to use well-water.

A typical outbreak of typhoid fever, traced to a specific pollution of the water-supply, is reported by Thresh.[3] A number of typhoid cases occurred in the small town of Halsted in the northwestern

[1] Schüder: Ztschr. f. Hyg., 1901, 38, p. 343. Schüder's statistics on their face convey a somewhat exaggerated idea of the frequency of water infection, since the larger and more explosive outbreaks are mainly due to water and are the ones ordinarily placed on record, while small epidemics and isolated cases are less likely to be deemed worthy of report. Although the water epidemics are most conspicuous, it is noteworthy that the mortality from typhoid fever in the United States at the present time is greater in rural communities than in cities.

[2] Weichselbaum: Weyl's Handbuch d. Hyg., 1900, 9, p. 436.

[3] Thresh: Lancet, 1897, 1, p. 687.

part of the county of Essex, England. Investigation showed that at the time of the outbreak there was on the outskirts of the town a public drinking-fountain which was fed by subsoil water, the surplus water from this fountain being piped to the cottage of a workman in the town. Above the source of the spring supplying the fountain, and near the top of a hill, an isolation hospital had been recently erected. A short time after the first patient ill with typhoid fever had been placed in the isolation hospital, the man residing at the cottage supplied with water from the fountain was attacked by typhoid fever. The only likely source of the disease was the drinking-water, since this man had not been in communication with the family in which typhoid fever had developed, and had not been away from the town for over two months. A little later four children developed typhoid fever, and all of these acknowledged that they had drunk more or less frequently at the fountain in passing. About two weeks later another child was attacked with typhoid fever, and although four other children in the house in which this child lived attended the same school, only the one affected had drunk the fountain water. On further inquiry the pipes conveying the water to the fountain were found to cross the roadway and pass underneath the sewer leading from the isolation hospital; in fact, they were almost in contact with the sewer-pipe. The water-pipes were ordinary agricultural drain-pipes, while the sewer-pipes were earthenware with plugged joints, and the latter were so damaged that during heavy rains any leakage from the sewer could enter directly into the water-pipes. Bacterial examination of the water showed not only the presence of B. coli, but also of an organism which, by all the tests then applied, resembled very closely the typical typhoid bacillus. There were no other likely sources of infection in this neighborhood.

Besides such specific cases it has also happened repeatedly that a city on changing from a polluted to a pure water-supply has experienced an immediate reduction in the prevalence of typhoid fever. The city of Albany, New York, had its typhoid death-rate diminished to about one-third as a result of the introduction of a system of sand filtration. In Vienna the abandonment of the polluted Danube River water was followed by a decline in the annual typhoid death-rate from over 100 to about 6. In the city of Paris, which at times has been obliged to eke out its insufficient

supply of spring-water with the highly polluted water of the River Seine, it has been observed that a miniature epidemic of typhoid springs up in that district of the city temporarily supplied with the Seine water and follows from one part of the city to another the course of the impure water as it is turned now into the pipes of one section, now into those of another.

The largest explosive outbreaks of typhoid fever in the United States due to water infection have been those at Plymouth, Pennsylvania[1] (1885, 1104 cases, 114 deaths), Ithaca, New York[2] (1903, 1350 cases, 82 deaths), Butler, Pennsylvania[3] (1903, 1346 cases, 111 deaths), and Salem, Ohio.[4]

Experiments have shown that when water freezes the great majority of typhoid bacteria that it contains are immediately destroyed. Those that survive die off progressively. According to Park, not one in a thousand lives in ice longer than one month, and at the end of six months all are dead. The use of ice is therefore not so dangerous as the use of the water from which it is formed. Relatively few epidemics of typhoid fever have been proved to be due to the use of ice. Convincing evidence, however, that ice infection does sometimes occur is given in a report of Hutchings and Wheeler,[5] who showed that the use of ice in the St. Lawrence State Hospital near Ogdensburg, New York, was followed by an epidemic of thirty-nine cases. The ice was cut seven months before its use from the St. Lawrence River about three miles below the point where the Ogdensburg sewage entered the river. Living typhoid bacilli were isolated from samples of the melted ice examined after the breaking out of the epidemic.[6]

(*b*) *Milk.*—Some outbreaks of typhoid fever due to contaminated milk perhaps owe their origin to the use of polluted water for rinsing cans, bottles, and other utensils employed in the collec-

[1] First Ann. Rep. State Bd. of Health and Vital Statistics of Pennsylvania, 1886; see also Sedgwick: "Principles of Sanitary Science and Public Health," pp. 200–206.

[2] Jour. Amer. Med. Assoc., 1903, 40, pp. 781, 848, 913; Jour. New Eng. Water-Works Assoc., 1904, 18, p. 431.

[3] Jour. Amer. Med. Assoc., 1903, 41, p. 1476; Eng. News, 1903, 50, p. 574; Twentieth Ann. Rep. of Penna. State Bd. of Health, 1904.

[4] Jour. Amer. Med. Assoc., 1920, 75, p. 1498.

[5] Hutchings and Wheeler: Amer. Jour. Med. Sci., 1903, 126, p. 680.

[6] See also W. H. Park: "The Importance of Ice in the Production of Typhoid Fever," Jour. Amer. Med. Assoc., 1907, 49, p. 731.

tion and transportation of the milk, a few drops of water being inadvertently left in the vessel in which the milk is placed. Other outbreaks are caused by direct contamination of the milk through the agency of persons suffering from mild or ambulant cases, or of chronic germ-carriers engaged in processes that entail possible contact. The clue to the origin of milk epidemics is usually afforded by the development of cases of the disease along the route of a particular milkman, while at the same time neighboring families served with milk from other sources remain free from infection. Since typhoid bacilli, in contrast to their behavior in water, are able to multiply in milk, the establishment of creameries in which the custom prevails of mingling milks from many different farms increases the peril of diffusion, since milk from a single source may contaminate the entire output of a creamery. Milk epidemics are often mild in type and affect a disproportionally large number of women and children. Butter made from contaminated cream is a possible vehicle for typhoid infection; experiments in fact have shown that typhoid bacilli introduced into butter in large numbers can survive for as long as three and one-half months. There is some epidemiologic evidence of infection from butter.

(*c*) *Other Food Substances.*—Besides dairy products, certain other foods that are usually consumed in a raw state may be the means of conveying the disease. Oysters and other shellfish have come into particularly bad repute in this respect within recent years, for the reason that a number of typhoid epidemics in Great Britain and the United States have been found to be due to the eating of oysters grown near sewer outfalls or placed to "fatten" in the polluted waters of estuaries or creeks.[1] Water-cress, lettuce, radishes, or any vegetables or fruits which are liable to come in contact with contaminated water or are sprayed with excrementitious matter are also capable of conveying infection.

(*d*) *Flies.*—Contamination of various articles of food by the wandering house-fly has long been a recognized possibility, but its importance and relative frequency have only recently become known. The severe visitation of typhoid fever in the camps of American soldiers during the Spanish-American War is in large

[1] For a concise summary of the data upon oyster infection, with full bibliography, see an article by G. W. Fuller: Journal of Franklin Institute, Aug., 1905, p. 81.

part plausibly attributed to infection of this character.[1] Lime was used for disinfecting the latrines in these camps, and flies with whitened feet were subsequently seen walking over the food on the mess-tables. Not only may bacilli stick to the legs and wings of these insects, but if swallowed by a fly they may survive the passage of the alimentary tract. Typhoid bacilli have been isolated from house-flies captured in houses in Chicago in the neighborhood of badly kept privy vaults used by typhoid patients, and it has been shown experimentally that living bacilli may remain in or upon the body of flies for as long as twenty-three days after infection.[2] It is possible that other insects, such as cockroaches, may in a similar way act as mechanical carriers of typhoid bacilli to food-substances.

(*e*) *Dust.*—Typhoid bacilli may conceivably sometimes be inhaled with contaminated dust, but according to our present knowledge such mode of infection must be extremely rare. Cases formerly attributed to air-carriage may perhaps be more reasonably ascribed to the agency of flies. Food, however, may be contaminated by means of sand or dust storms in infected localities. This seems to have been an important factor in the causation of typhoid fever among the British troops in the South African War.

(*f*) *Contact.*—Under the head of contact may be included those cases of infection due to particularly direct and immediate transfer from the infected to the healthy. The liability of those who nurse typhoid fever patients to contract the disease is well known. Since a drop of urine or a small particle of fecal matter may contain many thousands of typhoid bacilli, it is safest to regard the immediate surroundings of typhoid patients as contaminated, and to institute appropriate precautions. In some cases contact infection may occur when the patient is still in the incubation period of the disease.[3] Certain individuals have been found to discharge typhoid bacilli in the stools or urine for as long as eight, eleven, or even twenty-five days before the malady has become clinically manifest. Children seem to suffer not infrequently from a mild

[1] Abstract of Report on Origin and Spread of Typhoid Fever in U. S. Military Camps during the Spanish War of 1898. Reed, Vaughan, and Shakespeare, Washington, D. C., 1900. See also Report upon Typhoid Fever in Winnipeg, E. O. Jordan, 1905 (published by the City Council).

[2] Alice Hamilton: Jour. Amer. Med. Assoc., 1903, 40, p. 576.

[3] Klinger: Arb. a. d. k. Gesund, 1909, 30, p. 584.

ambulant, unrecognized form of typhoid (Brückner). In almost countless ways typhoid bacilli may find access to the alimentary tract of attendants or associates. Cases of direct infection are unquestionably more common than is generally recognized. Many cases due to secondary or contact infection follow in the wake of every large epidemic. The existence of "typhoid carriers" accentuates the danger of contact infection.

Typhoid Carriers.—The term "chronic typhoid-carrier" is ordinarily applied to those persons in whose bowel or bladder discharges typhoid bacilli can be detected at least six months after convalescence. The proportion of typhoid patients who become "chronic" or "permanent" carriers is not known owing to the difficulty of examining the secretions repeatedly and with proper methods over a sufficiently long period. Different investigators have reported proportions ranging from 0.5 per cent. up to 11.6 per cent.

Bacilli are sometimes discharged by carriers, as shown by exact bacteriologic control, over periods of several years, and probably in some cases throughout the whole of a long life. Typhoid germ-carriers are unquestionably responsible not only for occasional contamination of water and milk, but for direct contact infection of the immediate associates and comrades. Soper[1] brought to light the remarkable instance of a New York cook who was the innocent cause of some 26 cases of typhoid fever in seven different families. In the majority of typhoid carriers, as already pointed out, the bacilli seem to have established themselves in the gall-bladder. Attempts to secure an internal disinfection and prevent the continued elimination of bacilli have so far been unsuccessful.

The importance of the typhoid carrier in spreading the disease can hardly be overestimated. Intensive study of typhoid fever prevalence in certain districts in Germany has shown that an extraordinarily large proportion of cases can be indubitably traced to typhoid carriers. In one typhoid-ridden village in Trier it was found that 26.6 per cent. of all cases originated from contact with carriers, while in 15.6 per cent. more it was doubtful whether the infection came from carriers or from definite typhoid cases. Mayer[2] found that in a certain district in Bavaria carriers were responsible

[1] Soper: Jour. Amer. Med. Assoc., 1907, 48, p. 2019.
[2] G. Mayer: Centralbl. f. Bakt., Orig., 1910, 53, p. 234.

for 32.3 per cent. of the typhoid cases. This author gives a remarkable "genealogical tree" of 196 cases traced to a single case. Thirteen carriers appear among the cases. In the rural districts of Alsace-Lorraine and the Palatinate measures directed against the spread of the disease by contact and carriers were successful in five years in reducing the annual number of cases from 3487 to 1648. A comprehensive summary of the typhoid-carrier question has been given by Ledingham and Arkwright.[1]

The proportion of typhoid carriers in the general population is not definitely known owing to the technical difficulties already mentioned. It is probably quite different in different localities and doubtless depends largely upon the prevalence of current and past typhoid infection. Havens and Dehler,[2] using especially effective methods, found among 1076 dairy workers in Alabama 39 typhoid carriers, 13 carriers of B. paratyphosus **A** and 3 B. paratyphosus **B.** This makes a total of about 5 per cent. of positive results, a considerably higher proportion than that obtained in other localities (*e. g.*, Washington, D. C.) by less searching methods.

Control of carriers on the basis of bacterial tests is made a difficult matter by the fact that in some cases the excretion of typhoid bacilli is intermittent. Kayser records one case where the primary attack occurred in July, 1904. Two examinations made in August, 1904, and one in October, 1905, were negative; but in December, 1905, typhoid bacilli were found in the dejecta. Intermittent carriers have been reported by a number of other observers, and the problem of their detection and supervision is especially serious. A majority of typhoid carriers, but not all, give the Widal reaction, and in most cases the opsonic index is abnormally high.

Immunity.—An unmistakable attack of typhoid fever confers a certain degree of immunity, although instances of two or even more attacks in the same individual are not unknown. The cutaneous reaction is probably of value in measuring immunity against typhoid fever.[3] If a preparation of a killed culture of the typhoid bacillus is rubbed on the abraded skin, a specific reaction occurs in

[1] Ledingham and Arkwright: "The Carrier Problem in Infectious Diseases," London, 1912; see also Nichols: "Carriers in Infectious Diseases," Baltimore, 1922, and a valuable monograph by Garbat (Monograph 16, 1922, Rockefeller Inst. for Med. Res.).

[2] Havens and Dehler: Jour. Preventive Med., 1927, 1, p. 359.

[3] Gay and Force: Univ. of California Publication, Pathology, 1913, 2, p. 127.

most persons with a definite history of typhoid fever and in those recently vaccinated against the disease. It seems likely also that certain mild forms of intestinal disturbance, which are in reality light but unrecognized cases of typhoid fever, afford a certain protection against the severer forms of the disease. The relative freedom from typhoid shown by the permanent residents of a city having an impure water-supply, as compared with the susceptibility of the stranger within the gates, may perhaps be explained in this way.

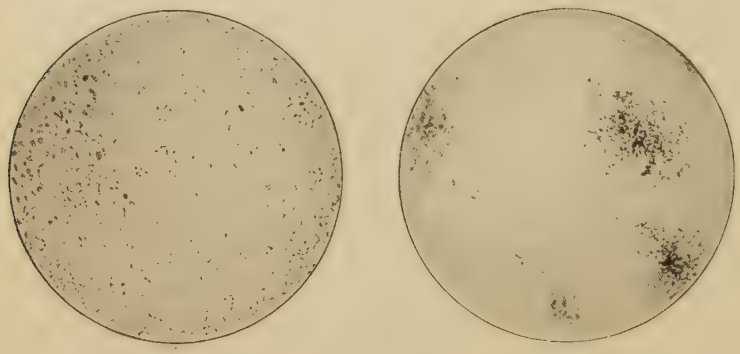
Experiments with animals have shown that it is possible to obtain a high degree of immunity in rabbits and guinea-pigs against intraperitoneal inoculation. This can be brought about by gradually increasing the amount of intraperitoneal bacterial injection, the animals after appropriate treatment being able to withstand amounts many times larger than the dose that would have been fatal at first. The immunity is associated with the acquisition by the body-fluids of a specific germicidal power. When typhoid bacilli are introduced into the peritoneum of an immunized animal, they are speedily dissolved and disintegrated by the peritoneal fluid in a manner not observed in a normal animal (Pfeiffer's phenomenon). Pfeiffer and Kolle[1] also showed that the simultaneous injection of immune serum and typhoid bacilli into a normal animal led to a similar destruction of the bacilli, while control animals that were inoculated with bacilli alone died. The development of this germicidal property in the body-fluids is due to antigenic substances contained in the bacterial cells. The injection of filtered broth cultures does not impart any bacteriolytic power to the serum.

The nature of the germicidal action and the mechanism concerned in it are described elsewhere (see p 159.). The bactericidal power of immune serum may be observed in test-tube experiments as well as in animal inoculation. To a mixture of normal serum and typhoid bacilli, graduated quantities of the serum of an immunized animal or typhoid convalescent are added and allowed to stand for a definite period. Plates are then made and the dilution noted at which bactericidal action is manifest. There is no constant relation between the bactericidal power of a serum in a test-tube and that of the same serum in the animal body. At the height of the disease when the serum shows its highest potency in the test-tube experi-

[1] Pfeiffer and Kolle: Ztschr. f. Hyg., 1896, 21, p. 203.

ment, the same serum mixed with bacilli and introduced into the animal body often exerts little or no germicidal effect.

Agglutination.—Not only a germicidal substance, but also, as is well known, an agglutinating substance, makes its appearance within the body of inoculated animals. In other words, the blood-serum of animals which have been injected with typhoid bacilli possesses the property of clumping or agglutinating suspensions of typhoid bacilli (Fig. 74). This reaction may be observed either with the microscope or, under suitable conditions, in a small test-tube with the naked eye, since the clumps of agglutinated bacteria form visible flocculent particles which eventually settle to the bottom of the tube as a fine sediment.



A *B*

Fig. 74.—Application of the serum-reaction to typhoid bacilli. *A* shows the distribution of the bacilli before the reaction. *B* shows clumping of the motionless bacilli after mixture with the serum of a case of typhoid fever.

The agglutination phenomenon (Gruber-Widal reaction) has been utilized extensively for the purpose of diagnosing typhoid fever in man. The fact that the serum of typhoid patients in rather high dilutions causes agglutination in the vast majority of instances (over 90 per cent. in the fourth week of the disease), while the serum from normal individuals and from those suffering from other diseases than typhoid fever does not possess the same power, has been taken advantage of, to facilitate the recognition of clinically obscure cases of the disease. In making the test the serum should be mixed with an authentic culture of typhoid bacilli in a dilution of not less than 1 : 50, since serum from normal individuals may produce agglutination in lower dilutions. Although the microscopic reaction is more delicate, that is to say it can be observed in

lower dilutions, the macroscopic test is less open to error even for experienced observers. Beginning agglutination may often be seen in two hours, and may be confirmed by the twenty-four hour appearance. As has been already pointed out, some cases clinically resembling typhoid fever may be caused not by the true typhoid organism, but by some member of the paratyphoid group, and in such cases agglutinative power for the typhoid bacillus may be lacking. The ability of the blood-serum to agglutinate is usually manifested as early as the fifth day, but sometimes does not appear until much later. The agglutinative power does not vanish soon after the blood is drawn, as does the germicidal property, but may persist with slightly diminished intensity for many months. Dried blood and blood-serum retain the capacity for agglutination, and the use of dried blood-serum in municipal laboratory work is very general (Wyatt Johnston). Many difficulties and sources of error beset the application of the agglutination test in practice. The agglutination reaction probably has neither more nor less diagnostic value than any of the cardinal clinical symptoms of typhoid fever. Its presence or absence does not by itself permit a positive or negative diagnosis. Some strains of genuine typhoid bacilli are inagglutinable, and, in general, freshly isolated cultures are less sensitive than those that have been under cultivation for some time. If broth cultures are used, an inexperienced observer might be deceived by the spontaneous clumping that sometimes occurs in this medium; a suspension in physiologic salt solution of bacilli from an eighteen-hour-old agar culture is preferable. A series of mistaken conclusions is made possible by the occurrence of group-agglutinins (p. 183). Especially the serum of persons infected with B. paratyphosus **B** may agglutinate the typhoid bacillus, and the same is true of infections with B. proteus (p. 476). It is desirable to make parallel tests with the typhoid bacillus and B. paratyphosus **B**, although at present the treatment of a case could hardly be affected by the outcome. The instances in which the serum of an undoubted typhoid patient agglutinates the paratyphoid bacillus more strongly than the typhoid bacillus itself are so rare as to have little practical significance.

The agglutination test is utilized not only for the purpose of distinguishing typhoid fever from other diseases, but also for

differentiating the true typhoid bacillus from closely related organisms of the same group. The blood-serum from a typhoid patient may be used for this test, and serum may also be used from an animal (rabbit, goat) which has been inoculated with a typhoid culture of undoubted genuineness. To avoid sources of error due to the generation of a certain degree of agglutinative power for other organisms of the group, dilutions as high as 1 : 1000 must be used. The use of the agglutination test for identification is open to the practical limitation that the test may exclude organisms of great biologic similarity to the typhoid bacillus and possibly of similar pathogenic, if not agglutinin-producing, power.

Serum-therapy and Protective Vaccination.—Although Chantemesse,[1] of Paris, and some others have reported favorable results from treating typhoid patients with a specific serum, the majority of observers have found that the use of serum from animals inoculated with typhoid bacilli or their products has little or no effect upon the course of the disease. On the other hand, protective vaccination against typhoid fever has been markedly successful. The vaccines prepared by different experimenters are not precisely alike, but all contain bacterial cells or substances derived from them.

The method of antityphoid vaccination has thus far found its widest application in the protection of soldiers in the field. The conditions of camp life favor the spread of the disease to an astonishing extent. In the Franco-Prussian War 60 per cent. of the total German mortality was due to typhoid fever, there being 73,396 cases and 8789 deaths. In the Boer War the British Army had 31,000 cases and 5877 deaths. In the Spanish-American War the army of the United States, consisting of 107,973 men, had 20,738 cases and 1580 deaths, or nearly 1 case to every 5 men.

Radically different is the history of typhoid fever in a vaccinated army corps. In the British Army in India in 1910 the rate of typhoid attack was about one-sixth as great among the inoculated as among the uninoculated. Similar results were obtained in the German troops in South Africa in 1904–1907, although the difference was not quite so great.

Especially brilliant results have been obtained in our own army by the method of vaccination introduced by Russell and his coworkers. During the summer maneuvers of 1911 an army

[1] Chantemesse: Gaz. des Hôpitaux, 1898, 71, p. 397.

division of about 12,800 men occupied a camp at San Antonio, Texas, for four months. All of the men were inoculated, and only a single case[1] of typhoid fever developed in the entire command during this period. There was no doubt that typhoid fever existed in this neighborhood, since at least 19 deaths from this disease occurred in the city of San Antonio (population 96,614) during the four months covered by this report. In the first 60,000 inoculated men in the United States Government service only 12 cases of typhoid—1 fatal—occurred in the space of about three years. This is about one-fifteenth of the case incidence in a city like Boston, where water-supply and general sanitary conditions are good and typhoid fever is not common, but where the population is unvaccinated.

In the United States Army, in which typhoid vaccination has been compulsory since September, 1911, the disease has practically disappeared. In 1909 there were 173 cases and 16 deaths in a force of about 69,000 men; in 1912, only 27 cases and 4 deaths in about 83,000. From August, 1912, to August, 1913, there were only 2 cases. Many of these troops were engaged in maneuvers in Texas (1911–1912) and were associated more or less intimately with the civil population, among whom typhoid cases were occurring in considerable numbers. Owing to the conditions under which soldiers must live in the field or on the march, the likelihood of typhoid fever is great. In 1898 at the time of the Spanish-American War, 4422 cases of typhoid and 248 deaths occurred in a division of 10,759 men; among 12,801 vaccinated men under very similar conditions at San Antonio, Texas, in 1911, only 1 case developed.

Data compiled from the incidence of typhoid in the American Army during the World War show that there was 1 case for every 3756 troops and 1 death for every 25,641 troops who served between April 6, 1917, and November 11, 1918, while during the Spanish-American War there was 1 case among every 5 and 1 death among every 71 troops. From September 1, 1917, to May 2, 1919, there were 213 deaths from typhoid in the American forces among an average strength of 2,121,396 men. Had the Civil War rate prevailed among this number, the number of deaths would have been

[1] An individual who had not completed the necessary inoculation. The attack was a mild one. Kean: Jour. Amer. Med. Assoc., 1911, 57, p. 713.

51,133, and had the Spanish-American War rate prevailed the number would have been 68,164.

The method used by Russell[1] consists in giving three injections of the vaccine at intervals of ten days. The first injection comprises 500,000,000 killed typhoid bacilli suspended in salt solution, the second and third, 1,000,000,000 bacilli each.

The vaccine is prepared from an old culture with little or no virulence, but which yields an abundant growth on agar. After eighteen hours' incubation on a broad agar surface the culture is washed off with sterile salt solution and is killed by heating at 55° to 56° C. for one hour. The suspension is standardized by counting the number of cells it contains and then diluted so that 1 c.c. contains 1,000,000,000 bacilli. A preservative (0.25 per cent. tricresol) is added, and the purity of the suspension thoroughly tested by cultivation and animal inoculation. As a rule, the reaction following inoculation is not severe, although occasionally fever, chills, nausea, and some nervous symptoms are observed. No really serious or permanent injuries have been noted.

The use of a protective inoculation is especially desirable where definite danger of typhoid infection exists, as among hospital nurses and attendants, and specially among soldiers living under the unhygienic conditions of war-time.

THE DYSENTERY BACILLUS (BACILLUS DYSENTERIÆ)

The name dysentery is primarily a clinical term, and is applied to several diseases or pathologic conditions of the alimentary tract that exhibit similar symptoms, such as intestinal pain and blood in the stools; as a matter of fact, different kinds of dysentery exist, due to different causes. One variety of dysentery, the so-called amebic dysentery, is caused by a protozoön, and is considered elsewhere in this book (p. 555). A dysentery-like infection may sometimes be produced by members of the paratyphoid-enteritidis group. Another bacterial form of dysentery is also known, caused by certain bacilli closely related to the typhoid bacillus.

In 1898 the Japanese bacteriologist, Shiga,[2] while studying a severe epidemic of dysentery in Japan, could find no amebæ in the stools. He did, however, succeed in isolating a bacterium much

[1] Russell: Bost. Med. and Surg. Jour., 1911, 164, p. 1; Jour. Amer. Pub. Health Assoc., 1911, 1, p. 473; Jour. Amer. Med. Assoc., 1919, 73, p. 1863.

[2] Shiga: Centralbl. f. Bakt., 1898, 23, p. 599.

like the typhoid bacillus. This micro-organism possessed certain definite characters, was found in the stools in all cases of epidemic dysentery, and was agglutinated by the serum of dysenteric patients in high dilutions. *Shiga's bacillus* is today generally regarded as the specific microbe of one of the acute epidemic forms of dysentery most common in temperate climates.

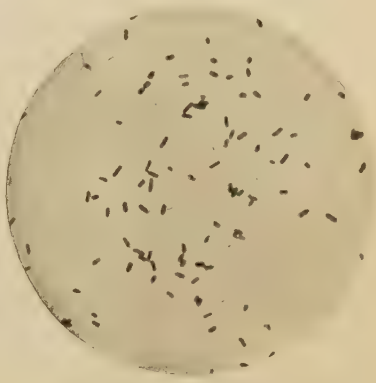


Fig. 75.—Bacillus dysenteriæ from agar culture. Fuchsin stain. Zettnow prep. (Kolle and Wassermann).

Characteristics of the Dysentery Bacillus.—Microscopically and in its staining reactions B. dysenteriæ is very much like B. typhosus (Fig. 75). Motility has been rarely observed, and most observers have failed to find flagella.[1] The growth on gelatin and agar and on potato resembles that of B. typhosus (Fig. 76). An alkaline reaction, however, is usually produced in milk following a slight initial acidity which may persist for a week or longer. Neutral red agar is not decolorized. Acid is produced in glucose broth. Indol is formed by some of the Flexner strains and not by others; it is not formed by strains of the Shiga type.

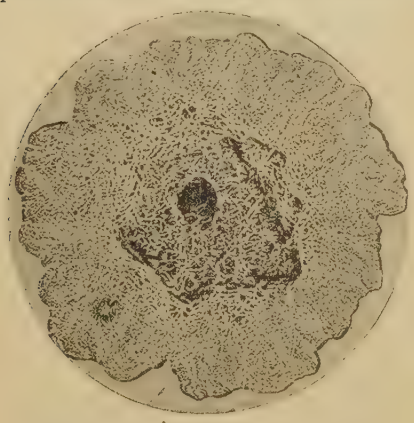


Fig. 76.—Bacillus dysenteriæ. Colony on gelatin, four days; × 20 (Doerr).

Kendall and Walker,[2] as the result of extended experience, give the following method for the isolation of B. dysenteriæ from stools: After plating on Endo medium (p. 326) small translucent colonies are transferred to mannite-litmus semi-solid medium, and incubated for twenty-four hours. Non-spreading growths in the medium are

[1] See, however, Goodwin, Park: "Pathogenic Bacteria and Protozoa," N. Y., 1905, p. 254.

[2] Kendall and Walker: Jour. Med. Res., 1910, 23, p. 481.

then to be examined further for agglutinative and other characteristics. The mannite furnishes a means for differentiating between the Shiga (non-mannite-fermenting) and Flexner (mannite-fermenting) types of dysentery bacilli. Acid, but no gas, is produced by the latter variety. B. coli, B. cloacæ, and B. proteus, on the other hand, form gas in the mannite medium. Agglutination with specific serum from either the Shiga or Flexner strain, in dilutions of not less than 1 : 200, must constitute the final test.

Varieties.—Bacilli identical with Shiga's bacillus in their morphologic, cultural, and agglutinative characters have been isolated in epidemics of dysentery in parts of Asia, Europe, and North America. In some cases, however, different although closely related bacteria have been found. Flexner,[1] when in Manila, isolated from dysenteric stools an organism at first supposed to be of the Shiga type, but later discovered to differ in agglutinative and other characteristics. Martini and Lentz,[2] as the result of an extensive study of bacilli isolated in epidemics of dysentery in different parts of the world, concluded that two types could be distinguished—one, the true Shiga bacillus, the other the Flexner-Harris organism. Many cultures that had been obtained from dysenteric stools were found to belong to the latter type. The two varieties differ not only in agglutinating reactions, but also in fermentive power: the Manila type breaks down mannite, with acid production, while Shiga's bacillus is unable to attack this substance. A mannite-fermenting dysentery-producing bacillus was found by Strong which differed from Flexner's bacillus in that it did not ferment maltose. Later a fourth type, sometimes known as the 4 type, was isolated by Hiss and Russell. Many other varieties have been reported by different observers. Otho[3] has even separated dysentery bacilli into fifteen groups, which have fermentation characters distinguishing them from one another. Further study of the varieties of dysentery bacilli have failed to show that the several types either possess much stability or are of practical significance. At the present time only the two main groups, non-mannite fermenting (Shiga) and mannite fermenting (Flexner) are usually taken into consideration. The latter, however, contains

[1] Flexner: Centralbl. f. Bakt., 1900, Abt. I, 28, p. 625.
[2] Martini and Lentz: Ztschr. f. Hyg., 1902, 41, pp. 540, 559.
[3] Otho: Philippine Jour. Sci., 1906, 1, p. 951.

a number (five or more) agglutinative types, a fact that complicates serological diagnosis. The Flexner bacillus is more common than the Shiga bacillus in sporadic cases of dysentery in children in this country.[1]

Pathogenicity.—As already stated, the serum of patients suffering from acute dysentery agglutinates one or another type of dysentery bacillus in high dilutions. This fact and the constant occurrence of the bacillus in the stools afford powerful arguments for a causal connection. Laboratory infection with a pure culture of the Flexner bacillus has occurred.[2] Apart from the inflammatory, sometimes ulcerative or diphtheritic lesions in the intestine (ulcerative colitis), the anatomic picture of dysentery presents little that is characteristic. The liver abscesses that are found, as a rule, in amebic dysentery are absent in the bacterial disease, one series having been reported of 1130 cases of bacillary dysentery without a single abscess. B. dysenteriæ is sometimes found in immense numbers in the dejecta, often in almost pure culture. It is found at autopsy in the mesenteric glands, but, as a rule, not in the spleen or other internal organs, nor does it commonly occur in the blood or urine. Bacterial dysentery is therefore not a septicemia but an infection localized in the alimentary tract, in this respect resembling Asiatic cholera rather than typhoid fever.

The spread of the disease is due to the more or less direct transfer of the specific bacillus from infected intestinal discharges to the alimentary tract of a fresh individual. Polluted water has been shown to be responsible for some epidemics. Dysentery, like typhoid fever, is a terrible scourge of armies. In general, the modes of dissemination in this disease are similar to those in typhoid fever, flies and finger contamination playing an especially important part. Direct contact is responsible for some cases. The danger from mild and unrecognized cases, and from convalescent germ-carriers, seems especially worthy of consideration.

Duval and Bassett[3] isolated B. dysenteriæ from the feces of forty-two out of fifty-three cases of summer diarrhea in infants. Subsequent investigators also found the dysentery bacillus in certain cases of infantile intestinal disturbances, especially those in which

[1] Davison: Bull. Johns Hopkins Hosp., 1920, 31, p. 225; Medicine, 1922, 1, p. 389.

[2] Lippincott: Jour. Am. Med. Ass'n, 1925, 85, p. 901.

[3] Duval and Bassett: Amer. Med., 1902, 4, p. 417.

there was mucus in the stools. Those cases with which B. dysenteriæ is associated do not appear to differ clinically from those in which it is not found. It is uncertain just what proportion of cases of infantile diarrhea are caused by the dysentery bacillus.[1]

Feeding animals with B. dysenteriæ is not generally successfu, in reproducing the symptoms or lesions of human dysenteryl although rabbits and guinea-pigs are highly sensitive to intravenous and intraperitoneal inoculation with living or dead bacilli, and die with symptoms of acute poisoning. Shiga has observed intestinal lesions in experimental animals similar to those of human dysentery.

Flexner and Sweet[2] have shown that the dysentery toxin is excreted in rabbits, and probably in man, by the large intestine. The selective action of the toxin upon the tissues rather than any local action of the bacilli themselves thus appears to be responsible for the inflammation and other local changes. When the toxin is introduced directly into the gut, no symptoms are produced, indicating that the toxin primarily affects the deeper cells rather than the surface of the mucous membrane.

Toxins, Serum Therapy, etc.—According to various investigators, both extracellular and intracellular poisons are obtainable from cultures of B. dysenteriæ. The death of a rabbit has been produced in twenty-four hours by intravenous injection of 0.02 c.c. of the filtrate of a seven-day broth culture, and extracts of agar cultures also possess toxic qualities. Olitsky and Kligler[3] have separated an exotoxin (neurotoxin) and endotoxin (enterotoxin) from cultures of the Shiga dysentery bacillus. Flexner,[4] upon injecting rabbits intravenously with the products of autolytic digestion of dysentery bacilli, observed the production of intestinal lesions analogous to those of human dysentery. In such a case the lesions would seem to be due to the elimination of the poison from the blood into the intestine and to the consequent contact of the poison with the intestinal tissues. Dopter[5] has shown that the products of the Shiga bacillus may cause paralysis in rabbits, the paralysis being referable to acute lesions in the ponto-bulbar region

[1] See Studies from the Rockefeller Institute for Medical Research, 1904, 2.

[2] Flexner and Sweet: Jour. Exper. Med., 1906, 8, p. 514.

[3] Olitsky and Kligler: Jour. Exper. Med., 1920, 31, p. 19; Ibid., 1923, 37, p. 767.

[4] Flexner: Jour. Exper. Med., 1906, 8, p. 514.

[5] Dopter: Ann. de l'Inst. Past., 1905, 19, p. 353.

or gray substance of the spinal cord, and Herter[1] has suggested that some cases of infantile spinal paralysis may be due to dysenteric infection.

Besredka has advanced the view that the immunity conferred by an attack of dysentery is due primarily to the local immunization of the intestinal mucosa rather than to the development of antibodies in the blood. There is some experimental evidence in its favor. The theory of local immunization has been extended to other infections.

Shiga and some other investigators have treated dysentery patients with the serum of horses injected with dysentery bacilli and their products, and have obtained favorable results, the mortality being reduced about one-half in the cases reported. A polyvalent serum, that is, one prepared by the use of several types of dysentery bacilli, is recommended by Shiga. The results are said to be more successful with Shiga bacillus infections than with Flexner bacillus infections and more successful in adults than in children. Theoretically and in its practical application, serum therapy in dysentery needs further study before it is likely to come into general use.

ALCALIGENES FECALIS

Alcaligenes fecalis or **B. fecalis alcaligenes**[2] closely resembles the typhoid bacillus morphologically and culturally, even to its growth on the Endo, Conradi-Drigalski, and malachite-green media. It has been found in feces and in water. The points of difference between it and the typhoid bacillus are: possession of one or more polar, instead of many peritrichal, flagella; more luxuriant growth on potato with a brown coloration; and distinct alkali production in mannite media and in milk or litmus whey. It fails to produce acid from dextrose and other carbohydrates. The view has been advanced that B. fecalis alcaligenes is merely a form of B. fluorescens non-liquefaciens, which has completely lost the function of pigmentation; but Klimenko,[3] who studied a series of cultures from different sources, found the two organisms to be distinct both culturally and in their reaction to the agglutination test. The cultures of his series were not pathogenic, or only slightly so, for

[1] Herter: "Bacterial Infections of the Digestive Tract," New York, 1907.
[2] Petruschky: Centralbl. f. Bakt., 1896, 19, p. 187.
[3] Klimenko: Centralbl. f. Bakt., 1907, I, Orig., 43, p. 755.

guinea-pigs, rats, and mice. This organism attracted considerable attention because of the assertion[1] that it and the typhoid bacillus were interconvertible, but further investigation has failed to confirm this claim, which seems to have been made on the basis of work with cultures not in a state of purity. Well-defined and constant differences separate the two bacilli.[2] Recent systems of classification place this organism in an independent genus (Alcaligenes).

Brucella melitensis

In 1887 Bruce,[3] while investigating a disease known as Malta fever, Mediterranean fever, or undulant fever, discovered a microorganism in the spleen which has since been proved to stand in causal relation to the affection. Undulant fever is particularly common on the island of Malta, but occurs also on other islands and on the shores of the Mediterranean, and has been occasionally reported from India, South Africa, the Philippines, and the West Indies. Several cases have come under observation in the United States, for the most part among persons recently returned from the Philippines. The disease has occurred in several localities in Texas.

Brucella melitensis is a very small coccus-like bacillus, about 0.5 μ or less in diameter, usually occurring singly or in pairs, though in cultures short chains are found (Fig. 77). A true bacillary form is present in cultures grown at 20° C. It loses the stain by Gram's method, does not ferment glucose, and renders milk slowly alkaline. On the ordinary culture-media it grows slowly without presenting especially characteristic features. Gelatin in not liquefied. In broth cultures there is no odor, and indol is not produced. Agar-plate colonies are small and transparent. A moist, transparent growth is formed on potato.

The generic name Brucella is commonly used for these organisms, although some bacteriologists would put them in the same genus with Alcaligenes fecalis with which they are undoubtedly closely related. A full discussion of the somewhat complicated nomenclature of this group has been given by Alice Evans.[4]

[1] Altschüler: Münch. med. Wchnschr., 1904, 51, p. 868; Doebert: Arch. f. Hyg., 1905, 52, p. 70.

[2] Gaehtgens: Arch. f. Hyg., 1907, 62, p. 152.

[3] Bruce: Practitioner, 1887, 39, p. 161.

[4] Alice Evans: Bull. Hyg. Lab., No. 143, 1925.

Undulant fever, as it affects man, is a disease of long duration and extremely irregular and undulating course, marked by shifting articular rheumatism and frequent and profuse sweatings. The case mortality is low (2 to 3 per cent.). The surest means of diagnosis, according to some writers, is the agglutination test.

Bassett-Smith is of opinion that a positive agglutination with a 1 : 30 dilution may be considered conclusive evidence of Mediterranean fever past or present.[1] On the other hand the agglutination test is considered unreliable by some observers, and the complement fixation test recommended in its stead.[2] In a large proportion of cases (82 per cent.) Br. melitensis has been found in the circulating blood. It can usually be obtained from the spleen at death or by spleen puncture during life. Like the typhoid bacillus, Br. melitensis is contained in the urine of many patients. It is less commonly found in the feces and in milk.

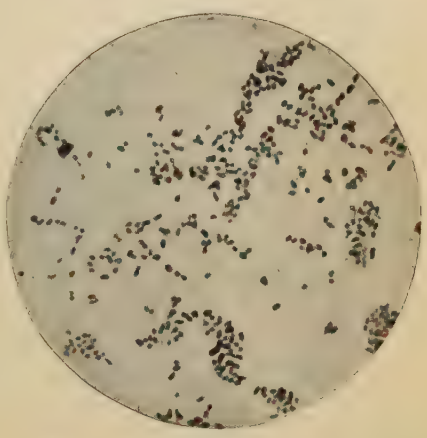


Fig. 77.—Brucella melitensis. Carbol fuchsin; × 1200 (Hicks).

There is no doubt that Br. melitensis is the cause of undulant fever, since several laboratory accidents have led to the infection, through small wounds, of persons working with pure cultures.[3] Monkeys, goats, and probably cows may be infected by injection and by feeding; the disease occurs spontaneously in goats and cows, and the urine and milk of these animals often contain the specific germ.

The mode of infection in undulant fever was for a long time quite obscure. The discovery that goats' milk often contains the specific micrococcus in large numbers at length gave the clue. It is stated that about half the goats in Malta are infected with Mediterranean fever, and that one-tenth are constantly passing the parasite of this disease in their milk. Incidents like the following emphasize especially this source of infection: Sixty-five goats, all apparently

[1] Bassett-Smith: Brit. Med. Jour., 1902, 2, p. 861.

[2] Mohler and Eichhorn: Jour. Amer. Med. Assoc., 1912, 58, p. 1107.

[3] Reports of the Commission on Mediterranean Fever, London, 1906, Part 4, p. 104.

healthy, were shipped at Malta in 1905 on the steamship "Joshua Nicholson" for export to America. The goats' milk was drunk during the passage in large quantities by the captain and many of the crew, with the result that almost every one who drank the milk was struck down with Malta fever. Sixty of the goats (five having died) on arrival in America were examined and thirty-two found to give the agglutination reaction, while Br. melitensis itself was isolated from the milk of several of them.[1] As a result of preventive measures directed against the use of goats' milk in Malta the cases on that island in the latter half of 1906 dropped to one-tenth of what, judging from past experience, would have been their normal number.

It seems possible that dust, contact and, rarely, inoculation through the agency of biting or suctorial insects, may play some part in spreading infection, but many observers believe that the cases arising from all these causes combined are relatively very few in number. Sergent,[2] however, while admitting the predominance of milk infection, emphasizes the possibilities of infection by contact. Water is apparently not a vehicle of transmission.[3]

BRUCELLA ABORTUS

Infectious abortion of cattle, a disease said to rank second only to bovine tuberculosis in economic importance, has been traced to a short, non-motile, pleomorphic, gram-negative bacillus (Fig. 78). MacNeal and Kerr[4] were the first in this country to record the isolation and cultivation of this organism, although Bang had described it in 1897. Growth takes place slowly on the ordinary culture-media. Best results in isolation are obtained in media containing glycerol or serum, or in mixtures of gelatin and agar. No perceptible change is produced in milk, although the organisms multiply well in this medium. No acid is produced from dextrose. The growth on potato is glistening and honey-like, often resembling that of the glanders bacillus. In cattle the bacillus shows a predilection for the mucous membrane of the uterus, where the changes it pro-

[1] Report of the Commission on Mediterranean Fever, Part 6, 1907, p. 70.

[2] Sergent: Ann. de l'Inst. Past., 1908, 22, p. 225.

[3] The bacteriology and epidemiology of this disease are very fully discussed in the Reports of the Commission Appointed by the Admiralty, the War Office, and the Civil Government of Malta for the Investigation of Mediterranean Fever, under the Supervision of an Advisory Committee of the Royal Society, Parts I–VII. London, Harrison & Son, 1905–07.

[4] MacNeal and Kerr: Jour. Infect. Dis., 1910, 7, p. 469.

duces give rise to abortion. One abortion may be followed by a second; rarely, by a third. Artificially the disease may be produced by way of the digestive tract and vagina as well as by subcutaneous inoculation. Guinea-pigs may be infected and abortion induced. Both the agglutination and complement fixation tests are used in diagnosis.

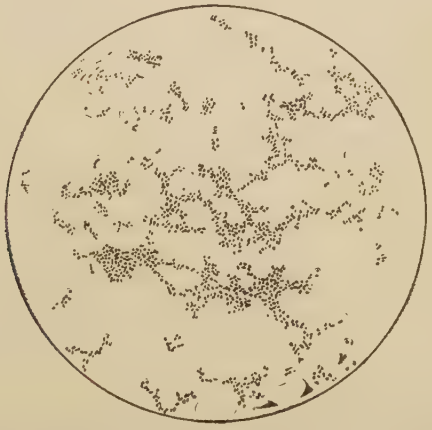


Fig. 78.—Brucella abortus. Eight-day culture. Carbol-fuchsin (Mohler and Traum, Twenty-eighth Annual Report, Bureau of Animal Industry, U. S. Department of Agriculture, 1911).

Relationship between Br. melitensis and Br. abortus.—The causative organisms of abortion in cattle and of Malta fever in man are very closely related, but not identical.

The delicate agglutinin absorption test shows that the majority of bovine strains fall into a single serological group separate from the strains isolated from man and from the goat. The present tendency is to regard the Brucella abortus type as a variety of the Malta fever organism—Br. melitensis var. abortus. Besides the serological difference the cells of the bovine type are thought to be slightly longer than those from human or caprine sources and to be somewhat less virulent for monkeys. There is now no doubt that Br. abortus is definitely pathogenic for man, apparently the first authentic instance being reported in Baltimore in 1924.[1] At least twenty cases have been recorded in the United States in which the type of infection was determined by the agglutinin absorption test.[2] In other countries there are records of cases of "Malta fever"

[1] Keefer: Bull. Johns Hopkins Hosp., 1924, 35, p. 6.

[2] Alice Evans: Jour. Amer. Med. Assoc., 1927, 88, p. 630.

in which infection could not be traced to any recognized source in goats. Among the sources of infection with Br. abortus may be contact with infected animals (cattle or swine) in slaughterhouses or elsewhere, or the drinking of raw milk from cows that had recently aborted. It is not known to what degree if at all Brucella abortus is concerned in cases of human abortion.

CHAPTER XIX

THE BACTERIA OF HEMORRHAGIC SEPTICEMIA;[1] BACILLUS PESTIS; BACTERIUM TULARENSE

Parasites. Non-motile, polar-staining. Gram-negative. Do not liquefy gelatin.

The term hemorrhagic septicemia was applied by Hueppe in 1886[2] to a group of highly fatal infectious diseases of the lower animals in which large and small hemorrhagic areas are found in the subcutaneous tissues, serous membranes, muscles and lymph-glands, and throughout the internal organs. In this class belong especially the affections described as swine plague (Ger., *Schweineseuche*), fowl cholera, and rabbit septicemia; with these is also to be ranked a disease of cattle described under a great variety of names (Wildseuche, Rinderseuche, Barbone, septic pleuropneumonia, pneumo-enteritis, etc.).

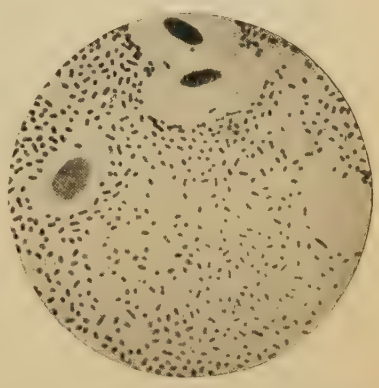


Fig. 79.—Bacillus of hemorrhagic septicemia in blood of a bird. Fuchsin stain (Kitt prep.) (Kolle and Wassermann).

The bacteria that are found in these widespread and important epizoötic diseases present many points of resemblance. They are short, non-motile bacilli, with a marked tendency to bipolar staining (Fig. 79). They are decolorized by Gram's method and do not form spores. Growth on gelatin is at best scanty, and liquefaction never occurs. In milk the reaction is usually slightly acid without coagulation. On acid potato, as a rule, no growth results. The names of B. avisepticus, B. bovisepticus, and B. suisepticus have been bestowed upon the cultures obtained respec-

[1] The generic name Pasteurella is used by some writers for the bacteria described in this chapter.

[2] Hueppe: Berl. klin. Wchnschr., 1886, vol. 23.

tively from fowls, cattle, and swine. These cultures derived from different sources are very similar, and in most cases no material difference can be detected in morphologic and cultural characters. The pathogenicity of the several strains is usually high, and, although variations in pathogenic power have been observed, most of the small laboratory animals and the common domestic animals succumb to inoculation. Fowls have been immunized against fowl cholera by cultures of the "bacillus of rabbit septicemia" (Kitt[1]), and Voges[2] produced in fowls a disease resembling fowl cholera by feeding them with cultures of the swine plague bacillus. There are many other facts that speak for the very close relationship, if not the identity, of the organisms found in the various forms of hemorrhagic septicemia. The name Bacillus pleurisepticus has been suggested as a unifying designation. So far as known, this disease or group of diseases is not communicable to man. Closely related to the bacteria of hemorrhagic septicemia are B. pestis, the bacillus causing the plague or "black death," and Bact. tularense.

Bacillus pestis

During the middle ages the plague prevailed extensively throughout Europe. The narrow, dirty streets and rat-infested dwellings of the walled towns, then becoming densely peopled, seem to have been highly favorable to the spread of the disease, and in some districts whole populations were carried off by the scourge. Hecker, a reliable authority, estimates that 25,000,000 persons, or one-quarter of all the inhabitants of Europe, perished in "The Great Mortality" or "Black Death" of the fourteenth century (1348–49). Few diseases have left so deep a mark on general literature. The Decameron of Boccaccio purports to be a collection of stories told by a company of ladies and gentlemen driven by the plague to take refuge in a country house outside the walls of Florence, and one of the most vivid descriptions of the plague ever written is from Boccaccio's pen. Defoe's famous "Journal of the Plague Year," although a fictitious narrative,[3] gives a realistic and essentially true picture of the devastation of London in 1665 by an outbreak of the dreaded "Black Death," in which 70,000 persons perished. Commerce and industry were then largely suspended

[1] Kitt: Kolle and Wassermann, Handbuch, 2, p. 559.

[2] Voges: Ztschr. f. Hyg., 1896, 23, p. 149.

[3] Defoe was only four years old in the year of the Great Plague.

and thousands of persons fled for safety to the open fields about London.

For reasons that may be only partly conjectured the plague has had irregular periods of quiescence and recrudescence. Western Europe has been practically free from the plague since the middle of the eighteenth century, and the disease began its first great extension in modern times with its appearance in 1893 in Hongkong and 1896 in Bombay. During recent years the plague has caused terrible loss of life in British India. Official statistics show that in the period from 1896 to 1907 about 6,000,000 deaths were due to this disease. In October, 1899, a case was recorded at Santos, Brazil; this is thought to be the first occurrence of the plague in the western hemisphere. Other cases have since been reported in and about San Francisco, in parts of Mexico, and in Central America.

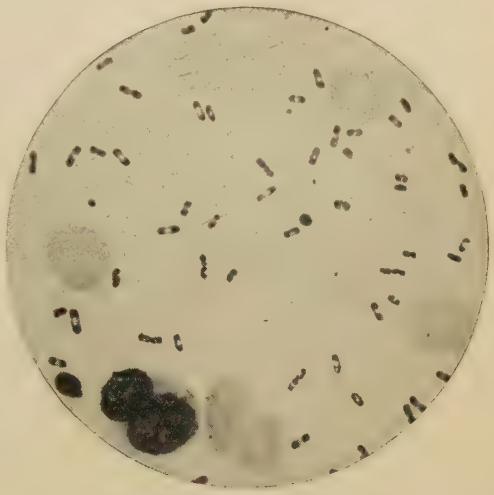


Fig. 80.—Bacillus pestis in smear from rat's liver, showing bipolar staining; × 720 (Wherry).

The specific bacillus of the plague (B. pestis) was discovered almost simultaneously by Yersin[1] and by Kitasato.[2] The germs are present in large numbers in the pulp of the young buboes,[3] which is described by one writer as a "purée" of bacilli.

Morphology.—Cover-slip smears made from the organs of a plague victim show a short, plump bacillus with marked bipolar staining (Fig. 80). Wright's modification of the Romanowsky staining method[4] is well adapted for staining the plague bacillus. The bacilli in body-fluids may occur in pairs, but long chains are rare. In broth cultures chains are the rule. Many morphologic

[1] Yersin: Ann. de l'Inst. Past., 1894, 8, p. 662.

[2] Kitasato: Preliminary Notice of the Bacillus of Bubonic Plague, Hongkong, 1894; Lancet, 1894, 2, p. 428.

[3] Inflamed and swollen lymphatic glands; hence the term "bubonic plague."

[4] Wright: Jour. Med. Res., 1902, 7, p. 138.

variations, coccus shapes and large rods, are found. Pale and swollen involution forms, often reaching a gigantic size, are very common, and, like the other variations from the short, polar-stained rod, occur both in fresh preparations from plague corpses and in cultures. In films made from animal fluids or organs, a capsule is usually difficult to demonstrate, but on nutrient agar, capsular substance may be produced abundantly (Fig. 81) (Wherry[1]). By Gram's method decolorization occurs. B. pestis is not motile. Spores have never been observed.

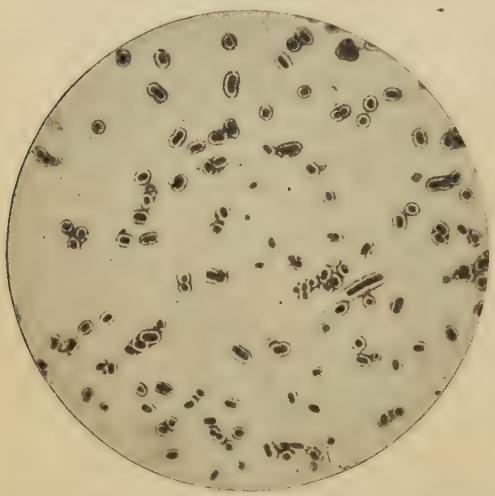


Fig. 81.—Bacillus pestis from agar culture, twenty-four hours, showing capsule; × 700 (Wherry).

Cultural and Biologic Characters.—Growth occurs on all the ordinary culture-media. Unlike most of the bacteria pathogenic for man, a temperature of 25° to 30° C. is more favorable than one of 37° C., and growth may even take place at a temperature as low as 4.5° C. Under all circumstances the colonies grow slowly and never attain a large size. Involution forms develop abundantly when the medium is dry. The addition of 3 to 4 per cent. of common salt to nutrient agar furnishes a medium that tends to produce involution forms so uniformly within the first twenty-four hours that the use of salt-agar has been recommended by Hankin and others as of diagnostic value. The colonies that develop on plates of ordinary nutrient agar and gelatin present a delicate, drop-like appearance with a round, granular center and a thin, granular, uneven margin (Fig. 82). Neither gelatin nor blood-serum is liquefied by the growth. On potato and in milk, multiplication is slow and scanty; milk is rendered slightly acid, but not curdled. No gas is produced in the presence of sugars, although a small amount of acid is formed from dextrose.

[1] Wherry: Jour. Infect. Dis., 1905, 2, p. 577.

One of the most characteristic cultural features is observed in the growth in broth. When the surface of this medium is covered with a layer of oil[1] and flasks are left after inoculation undisturbed for five or six days, long, delicate filaments are formed which hang down from the surface into the depths of the clear broth, like the stalactites that depend from the roof of a grotto. Not all cultures of B. pestis show the stalactite growth in equal degree, and, on the other hand, a similar formation has been observed in cultures of other bacteria; the stalactite formation, therefore, while highly characteristic, especially when broth is seeded directly from fresh plague buboes, is not specific.

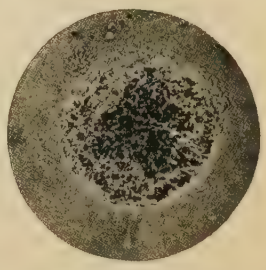


Fig. 82.—Colony of Bacillus pestis. Gelatin, forty-eight hours; × 150 (Wilson).

Toward various physiologic influences the plague bacillus does not exhibit marked resistance. Exposure to drying, particularly at the higher summer temperatures, speedily effects its destruction. The plague bacillus is also quite sensitive to the action of sunlight and chemical disinfectants. In general the life of B. pestis outside the animal body is precarious, and the bacillus seems to disappear speedily from soil, water, and buried cadavers.

Obscurity still prevails regarding the toxic products of the plague bacillus. The results of experiments with broth cultures have been taken to indicate that the toxic element is not so closely associated with the cell substance as in the cases of cholera and typhoid bacteria, but is able to diffuse to some extent into the surrounding liquid, like the diphtheria and tetanus toxins. On the other hand, no antitoxic immunity has been obtained.

Modes of Transmission.—Filth and poverty have long been recognized as factors contributing to the persistence and spread of the plague. It would appear that what are known as medieval surroundings, whether in Europe in the fourteenth century or in China and India in the twentieth, conduce to infection. Relatively close personal association is one of the conditions under which plague bacilli seem to be transmitted from the sick to the well. The sputum of plague patients suffering from the pneumonic type

[1] "Ghee," a kind of clarified butter, is often used by bacteriologists in India for this purpose.

of the disease is highly infectious. The "infectious droplets" (p. 441) discharged in coughing also make the immediate neighborhood of the pneumonic plague patient dangerous to others.

The outbreak of the pneumonic type of plague in Manchuria in 1911–12 illustrated the rapidity with which this type of the disease may spread and the difficulty of practically combating it. The risk to attendant physicians and nurses is very high.

There is evidence that in some epidemics of the plague, especially the bubonic type, transmission from one human being to another is not the way in which dissemination occurs. It was noted long ago by observers of epidemic plague that many outbreaks were accompanied by a remarkable mortality among rats. The discovery of the plague bacillus led to the further observation that the disease of rats was due to the same cause as that of man, and established the practically invariable association of rat plague and human plague. It is now known that rats[1] are highly susceptible to infection with B. pestis and may suffer spontaneously from epidemics under natural conditions. In San Francisco 14,184 rats were examined for plague between September 13, 1907, and January 14, 1908, and 192 were found infected. A chronic form of infection may exist among these animals, a circumstance that especially facilitates the dissemination of infection. Rats on shipboard are the means of carrying the disease from port to port, although human passengers may remain perfectly healthy. Many observers have expressed the opinion on epidemiologic grounds that plague is primarily a disease of rats and that man is only an incidental victim. In consequence of these relations the possible modes of conveyance of plague bacilli from rat to man have become a subject of peculiar interest. Both the feces and the urine of plague-infected rats sometimes contain plague bacilli, and it might be thought that contamination of the surroundings could lead either to cutaneous infection or to infection through the alimentary tract. Experiments[2] have shown, however, that close and continuous contact of plague-infected animals with healthy animals does not give rise to an epizoötic among the latter, *provided fleas are rigorously*

[1] At least three species, *Mus norvegicus*, the common brown sewer-rat; *Mus rattus*, the black house-rat and the ship-rat; and *Mus alexandrinus*, the Egyptian rat, are known to be capable of receiving infection.

[2] Report on Plague Investigation in India, Jour. of Hyg., 1906, 6, pp. 422–536; 1907, 7, pp. 323–476, and 694–985.

excluded. The share of fleas in carrying infection from rat to rat and from rat to guinea-pig has been established by convincing experiments. The blood of plague-infected rats often contains enormous numbers of plague bacilli, as many as 100,000,000 per cubic centimeter having been found. Rats freed from fleas do not become infected by mere contact with plague-infected animals. Monkeys, guinea-pigs, and healthy rats, on the other hand, may contract the plague if they are brought into the neighborhood of flea-infested plague rats. In the reports of the plague investigation in India the following experiment with monkeys is recorded. Two monkeys were placed in similar cages, so designed that fleas could not get in from the top, and the cages put into a flea-infested animal house where three guinea-pigs had died from plague inoculation a few days previously. One monkey-cage was surrounded by a layer of sticky fly-paper six inches wide, it having been found that the leap of which a rat flea is capable is not greater than about five inches; the other monkey was not so protected. After two nights in the animal house the cages were removed. Two fleas were caught on the unprotected monkey and five were found stuck on the fly-paper protecting the other monkey. The unprotected monkey developed a typical case of plague, while the monkey that had been surrounded by fly-paper remained healthy. Further experience in the experimental production of plague epidemics among animals has entirely confirmed these results.

It is possible to infect rats by feeding them with the carcasses of their plague-infected comrades. In rats infected in this way mesenteric buboes are most frequent. Cervical buboes, on the other hand, preponderate in naturally infected rats, in guinea-pigs infected by being placed in a plague-infected house, and in rats and guinea-pigs artificially infected by means of fleas. The examination on one series of 5000 naturally infected rats showed not a single case of mesenteric bubo. The conclusion seems justified, therefore, that rats in nature are not infected by feeding upon plague-infected material, but are infected, as a rule, through the agency of fleas.

There is much to support the view that the plague may be communicated to man also by the bite of the rat flea. The commonest rat flea, except in Northern and Central Europe, is *Pulex cheopis* (Rothschild), a species that will readily bite man. When abundant, it will bite man in the presence of its natural host. Rat

fleas have been found in large numbers on the legs of men who entered for a short time the rooms of a plague-infected house. The particular danger that is known to attach to sleeping in a plague-infected house is explicable from this standpoint. Ashburton Thompson,[1] who has made a careful study of four outbreaks of the plague at Sydney, concludes that the epidemiologic data in that locality harmonize best with the view that the rat flea transmits the disease from rats to man. Gotschlich[2] has found that in Egypt the winter plague epidemics are of the pneumonic type and are spread through human agency, while the summer plague cases are of the bubonic type and are due exclusively to rat infection. Kitasato has observed a similar seasonal incidence in Japan.

In California the native ground squirrels have proved highly susceptible to pest infection and, probably through the agency of these animals, the disease had in 1909 spread over a considerable area. Cases of plague in man due to squirrel infection have been reported.[3]

Pathogenicity for Man.—The belief that the bacillus called B. pestis stands in direct causal relation to plague received experimental confirmation from an accidental laboratory infection that took place in Vienna in October, 1898. A commission sent by the Vienna Academy of Sciences in January, 1897, to study the plague in Bombay, returned to Vienna some three months later bringing much material for observation and experiment. After the work with pure cultures of B. pestis had been in progress for some time, the man who cared for the animals under experimentation is supposed, when under the influence of drink, to have neglected some essential precaution. At all events he became infected; no other case of the plague existed in Vienna at the time. The physician, himself a member of the commission, and the two nurses who cared for this patient, all contracted the infection, and the physician and one nurse died.

Plague in man appears most commonly in two forms: the bubonic or glandular plague and plague pneumonia. In the bubonic type the symptom-complex is characteristic, and diagnosis on clinical

[1] Thompson, Ashburton: Jour. of Hyg., 1906, 6, p. 537.

[2] Gotschlich: Festschrift f. R. Koch, Jena, 1903.

[3] See Special Report on Plague on the Pacific Coast, Jour. Amer. Med. Assoc., 1907, 49, p. 2000; also McCoy and Wherry: Jour. Infect. Dis., 1909, 6, p. 670.

grounds is relatively simple. From the buboes, which may be either primary or secondary, bacilli may pass over into the blood; in fatal cases the bacteria often multiply in the blood extensively. A primary plague septicemia can also probably occur. There are sometimes subcutaneous hemorrhages. During the plague epidemics in the middle ages such hemorrhages seem to have been more frequent than at present, and the dark spots to which they give rise were the origin of the popular name of "the black death."

Plague pneumonia is usually fatal. In this variety of the plague the sputum may contain enormous numbers of plague bacilli. As a direct means of spreading contagion from man to man, plague pneumonia is by far the more dangerous type.

A primary infection of the skin with the plague bacillus sometimes occurs (cutaneous plague), but does not seem to be common. Cases of mild plague, the so-called *pestis minor* (compare "walking typhoid"), are met in some epidemics. The occurrence of intestinal plague in man has never been clearly established.

The usual entrance of the plague bacillus into the body is probably by way of the skin, the buboes originating in the neighborhood of the point of entrance. Infection through the tonsils and respiratory tract, especially from cases of pneumonic plague, can also take place. Infection by swallowing, on the other hand, if it occurs at all, is extremely rare.

Pathogenicity for the Lower Animals.—Many rodents, such as rats, mice, and guinea-pigs, are very susceptible to the plague. In California, however, from 20 to 70 per cent. of the full-grown rats (Mus norvegicus) are refractory when inoculated with highly virulent cultures. The California ground squirrels are much more susceptible than the rats. Certain species of monkeys are extraordinarily sensitive to subcutaneous inoculation, $\frac{1}{1000}$ to $\frac{1}{100}$ of a loopful of an agar culture being sufficient to produce a fatal septicemia. Both monkeys and rodents develop buboes and exhibit other features common to the plague in man. Rats and guinea-pigs may be infected by feeding, especially when large numbers of bacilli are administered. Cats are susceptible to artificial infection. Dogs, swine, cattle, and horses can be infected by inoculation of large doses, but apparently do not contract the disease spontaneously under natural conditions.

Owing to the close relation existing between plague in rats and in man the phenomena of rat infection have been especially studied. The diagnosis of plague infection in rats is practically important, and special procedures are in use for this purpose.[1] Experimental observers maintain that for purposes of diagnosis naked-eye examination by a competent observer is more satisfactory than microscopic examination alone.[2] Ledingham,[3] in observations upon spontaneous cases of rat plague, found that in some animals bacterial invasion of the spleen and liver is pronounced and is accompanied by extensive hemorrhages and congestion of the pulp sinuses and liver capillaries. In others, definite abscess formation in the spleen is far more frequent, while in the liver focal necroses may be very numerous. Externally the liver and spleen, especially the former, frequently present a granular and mottled appearance which is quite characteristic. Typical buboes are present in the great majority of cases (85 per cent.). Subcutaneous and internal hemorrhages are very common. In the experience of the investigators of rat plague in India an abundant, clear, pleural effusion is an important diagnostic sign.

The procedure followed in making a diagnosis of plague infection is to rub the material from a suspected case, human or rodent, on the freshly shaved belly of a guinea-pig and of a white rat. In true plague the animals usually die within ten days. In about one-third of the rats and in practically all guinea-pigs a local reaction is present at the site of inoculation. Guinea-pigs, as a rule, show a typical bubo, rats less commonly. The spleen is enlarged (in guinea-pigs two or three times the normal size) and studded with granules. The liver is granulated. Rats show the characteristic pleural effusions observed in natural infections. Plague bacilli may be isolated from the spleen, buboes or heart's blood.

Protective Inoculation and Immunity.—A method of protective inoculation against the plague devised by Haffkine[4] has been extensively practised in India. As usually prepared, "Haffkine's prophylactic" consists of broth cultures in which repeated crops (five to six) of the stalactite formation have been obtained by successive inoculations, shakings, and reinoculations. After about

[1] See Kister: Centralbl. f. Bakt., I, Orig., 1906, 41, p. 780.

[2] Wherry, Walker, and Howell: Jour. Amer. Med. Assoc., 1908, 50, p. 1165.

[3] Ledingham: Jour. of Hyg., 1907, 7, p. 359.

[4] Haffkine: Brit. Med. Jour., 1897, 1, p. 424.

six weeks the culture is killed by warming the flask of broth in the water-bath for one hour at 65°. The usual dose of the prophylactic is about 2 c.c., but larger quantities may be given, and the dose is generally proportioned to the age and size of the individual. Considerable success has attended its use: the proportion of vaccinated persons attacked is smaller than that of the unvaccinated in the same population, and those vaccinated persons who do contract the disease suffer from it in a comparatively mild form. The German Plague Commission has recommended an essentially similar procedure, namely, the injection of a two-day agar culture killed by heat. Kolle and Otto[1] have shown that in animal experimentation much better results are obtained with attenuated cultures than with killed bacteria, and Strong[2] has found on applying this method to man that a high degree of immunity to the plague may be produced by inoculation of an attenuated strain. Hueppe and Kikuchi[3] have reported favorable results in the active immunization of animals, from the use of the peritoneal exudates obtained from guinea-pigs that had been inoculated intraperitoneally with plague bacilli.

Yersin and Roux[4] have produced a serum that possesses some curative properties by injecting horses first with killed cultures, then with living cultures. The Yersin serum has considerable bactericidal power, but experiments have shown that its curative action is not due solely to bacteriolysis. There is evidence that it facilitates phagocytosis. No antitoxic effect has been observed. Many investigators have failed to secure favorable results with this serum, but, in the epidemic of plague at Oporto, Calmette and Salimbeni[5] employed it with success.

Tularemia. Bacterium tularense

This organism was discovered by McCoy and Chapin in a plague-like disease of the California ground-squirrel.[6] It is a minute gram-negative pleomorphic rod (Figs. 83 and 84) surrounded with considerable capsular substance when stained in smears from

[1] Kolle and Otto: Ztschr. f. Hyg., 1903, 45, p. 507; 1904, 48, p. 399.
[2] Strong: Philippine Jour. of Sci., 1906, 1, p. 181.
[3] Hueppe and Kikuchi: Centralbl. f. Bakt., 1905, I, Orig., 39, p. 610.
[4] See Metchnikoff: Ann. de l'Inst. Past., 1897, 11, p. 737.
[5] Calmette and Salimbeni: Ann. de l'Inst. Past., 1899, 13, p. 865.
[6] McCoy and Chapin: Jour. Infect. Dis., 1912, 10, p. 61.

animal tissues. The earlier workers succeeded in cultivating this bacillus only by the use of coagulated egg-yolk, but Francis[1] has obtained growth on other media such as blood glucose cystin agar

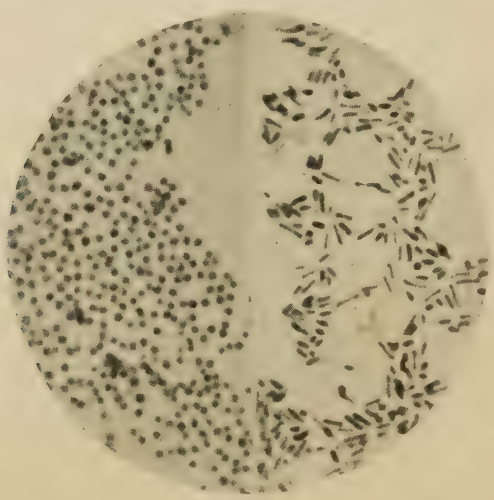


Fig. 83.—Bacterium tularense. Note change from coccoidal to bacillary form in 24 hours on fresh culture medium. (After Francis.)

(Fig. 85). Glucose, levulose, mannose and glycerol are fermented with production of acid but no gas. The first instances of human

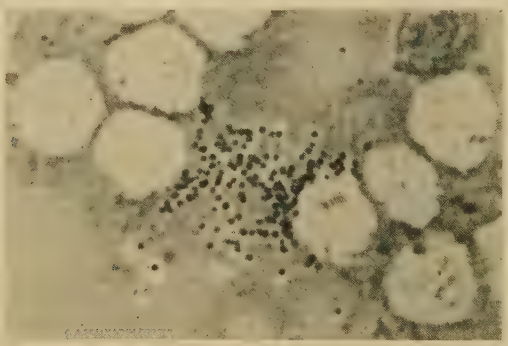


Fig. 84.—Bacterium tularense. Coccoidal form. Blood of rabbit. (After Francis.)

infection with this organism were three cases of eye infection recorded by Wherry and Lamb.[2] Later observations (see especially Francis[1]) have shown that human infections may occur through

[1] Francis: Bull. No. 130, Hyg. Lab., Wash.

[2] Wherry and Lamb: Jour. Infect. Dis., 1914, 15, p. 331.

handling infected rabbits and other rodents, or by the bite of a wood tick or a blood-sucking fly that has previously fed on a jack-rabbit infected with Bact. tularense (Fig. 86). The latter form of human infection is known locally in Utah as deerfly fever.[1] Infection with this organism has been contracted with amazing facility by laboratory workers, as many as eleven cases having been reported among the limited number of persons who have worked with this bacillus in the United States. It has been shown experimentally that the

Fig. 85.—Tularemia. Glucose cystine agar inoculated with heart blood of guinea-pig (A) and mouse (B). (After Francis.)

disease may be transmitted to laboratory rodents by the bite of infected lice or bedbugs.

It seems probable that infections with this organism have occurred not infrequently without recognition. Francis,[2] the leading student of this disease, has records of several hundred human cases in nearly all parts of the United States. Tularemia has also been reported in Japan.

Two clinical types of the disease are recognized, one the glandular or ulceroglandular type which is the more common, the other the so-called typhoidal type. Tularemia is rarely fatal. During the

[1] Francis and Mayne: Pub. Health Rep., 1921, 36, p. 1738. Francis has suggested the general name tularemia for all infections due to Bacterium tularense.

[2] Francis: Atlantic Med. Jour., 1927, 30, p. 337.

first week of illness blood cultures have given positive results on guinea-pig inoculation. Agglutinins are present in the blood in the second week of the disease and may persist in diminishing amounts for at least as long as 18 years after recovery. Agglutination is a

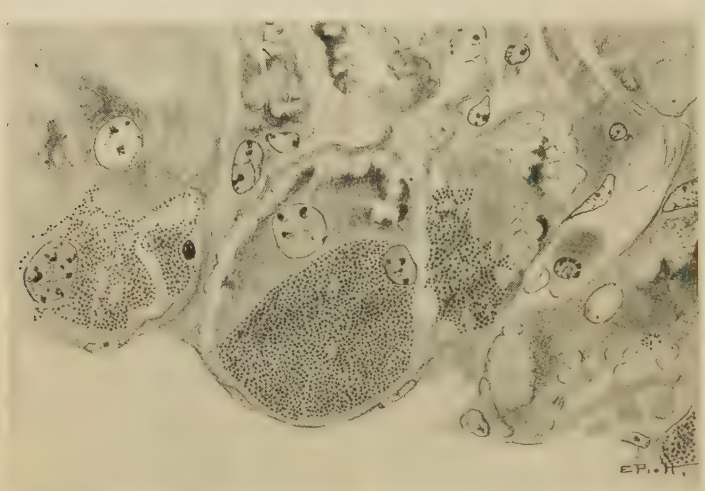


Fig. 86.—Bacterium tularense in hepatic cells of liver of mouse. (After Francis.)

reliable test for diagnosis, but reciprocal absorption tests for either Brucella abortus or B. melitensis should be made unless the clinical history is quite definite. One attack confers immunity.

CHAPTER XX

THE HEMOPHILIC BACTERIA

Genus Hemophilus. Minute rod-shaped cells, sometimes thread-forming and pleomorphic, non-motile, without spores, strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood-serum or ascitic fluid. Gram-negative.

The type species is Hemophilus influenzæ.

The hemophilic or hemoglobinophilic bacteria comprise a fairly large group of bacteria with more or less pronounced pathogenic qualities. Among these are Pfeiffer's bacillus (B. influenzæ) associated with influenza, the Bordet-Gengou bacillus (B. pertussis) found in whooping-cough, the Koch-Weeks bacillus and the Morax-Axenfeld bacillus which are associated with certain forms of conjunctivitis, and the bacillus of Ducrey, found in soft chancre. The presence of hemoglobin in the culture-medium is especially favorable for the growth of these organisms and is essential to the development of Pfeiffer's bacillus and the Koch-Weeks bacillus. Similar strictly hemophilic bacteria have been reported by D. J. Davis[1] and others in various pathological conditions.

The hemophilic bacilli are usually very minute, and are difficult to study both because of their small size and their far from robust growth on most culture-media.

The Pfeiffer bacillus may be distinguished from B. pertussis by its inability to grow on media devoid of hemoglobin even after several generations of cultivation, while B. pertussis after being grown in several transfers on a blood and serum medium will yield a slow growth of gray adherent colonies on plain agar. Many strains of Pfeiffer's bacillus (40 to 50 per cent.) produce indol,[2] a quality apparently lacking altogether in B. pertussis. Pfeiffer's bacillus also reduces nitrate to nitrite, whereas B. pertussis is

[1] D. J. Davis: Jour. Amer. Med. Assoc., 1915, 64, p. 1814.

[2] E. O. Jordan: Jour. Amer. Med. Assoc., 1919, 72, p. 1542; R. H. Malone: Indian Jour. Med. Res., 1920, 7, p. 519; T. M. Rivers: Bull. Johns Hopkins Hosp., 1920, 31, p. 50.

unable to effect this reaction.[1] The relation of the Koch-Weeks bacillus to Pfeiffer's bacillus is culturally very close and some investigators believe in a substantial identity of the two forms.

Hemophilus Influenzæ (Pfeiffer's Bacillus)

Morphologic and Cultural Characters.—The Pfeiffer bacillus is one of the smallest known pathogenic bacteria, rarely exceeding 1.5 μ in length and 0.3 μ in thickness. The ends of the cell are rounded, no capsule is present, and spores have never been observed; the bacillus is non-motile (Fig. 87). Many strains show a marked tendency to produce threads and other anomalous forms in cultures. Staining is best effected with a dilute (1 : 10) solution of carbol-fuchsin for five to ten minutes. Cultures of this organism were first obtained by Pfeiffer upon blood-agar, prepared either with human blood or the blood of other animals, such as guinea-pig or pigeon; and blood-agar remains to this day a practically indispensable medium for growing this bacillus. The hemoglobin of the blood is the essential ingredient, since growth occurs just as abundantly on agar moistened with a solution of hemoglobin as on agar prepared with whole blood. When the surface of blood-agar is smeared with influenza sputum and incubated at 37° C., with free access of oxygen, minute, rounded, discrete, translucent colonies may become visible in about eighteen hours. Transplanted to ordinary agar, development does not result, but on blood-agar further colony formation occurs. The colonies at the largest may reach the size of a small pinhead. If the culture be contaminated with other organisms, especially Staphylococcus aureus, the Pfeiffer bacillus colonies are considerably larger, more opaque, and of a grayish-white color. Even under favorable conditions artificial cultures soon die out, and in order to preserve vitality subcultures must be made on hemoglobin agar every four or five days; in this way the stock may be maintained indefinitely.

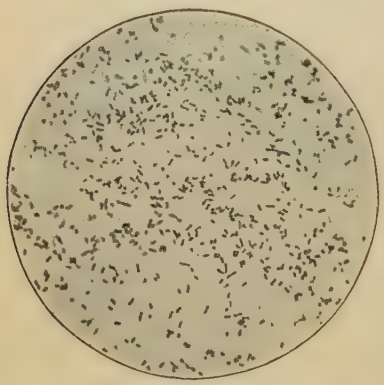


Fig. 87.—Hemophilus influenzæ; × 1000 (Král).

[1] Jordan: Amer. Jour. of Public Health, 1920, 10, p. 648.

Pfeiffer's bacillus requires for its growth the presence of two distinct substances, one a heat-labile substance, the so-called V factor which is present in yeast, carrot and potato juice and other vegetable extracts as well as in blood. The other substance—X factor—is typically represented by hemoglobin or its derivatives, is heat-stabile and is efficacious in very small amounts. Little is known about the chemical nature of the X and V factors.

If colonies of certain bacteria, *e. g.* staphylococcus, are present on a plate with Pfeiffer bacilli, colonies of the latter will develop most luxuriously in the neighborhood of the foreign colony. This "satellite phenomenon" is quite characteristic of the hemophilic bacteria and is evidently due to the diffusion into the medium of a growth-promoting substance—possibly the V factor—generated by the other bacteria.

Among the many attempts to devise a favorable medium for the cultivation of the Pfeiffer bacillus two have been especially successful.

(*a*) *Oleate Hemoglobin Agar.*—This medium[1] exercises a selective action since it allows the Pfeiffer bacillus (and some other organisms) to come to development, while many of the gram-positive cocci present in the sputum or the nasal mucus are inhibited by the sodium oleate. It is prepared as follows:

Hot nutrient agar = 90° C. (2 per cent. meat infusion agar; P_H 7.2–7.4)	94 c.c.
Sodium oleate (neutral, Kahlbaum's preferred; 2 per cent. solution in water; autoclaved)	5 c.c.
Suspension of red blood-corpuscles (sterile defibrinated human, sheep, or rabbit blood may be used. Remove the red cells from the defibrinated blood by centrifugation, pipeting off the supernatant fluid; restore the original volume with nutrient broth)	1 c.c.

The action of this medium is shown in Figs. 88, 89, and 90.

The use of oleate agar facilitates the isolation of the Pfeiffer bacillus and as compared with ordinary blood-agar plates gives a larger number of positive findings.

(*b*) *"Chocolate" Agar.*—A luxuriant growth of the Pfeiffer bacillus occurs on the opaque, dark brown medium obtained by adding a few cubic centimeters of blood to hot agar (at 90° to 95° F.).

[1] Avery: Jour. Amer. Med. Assoc., 1918, 71, p. 2050.

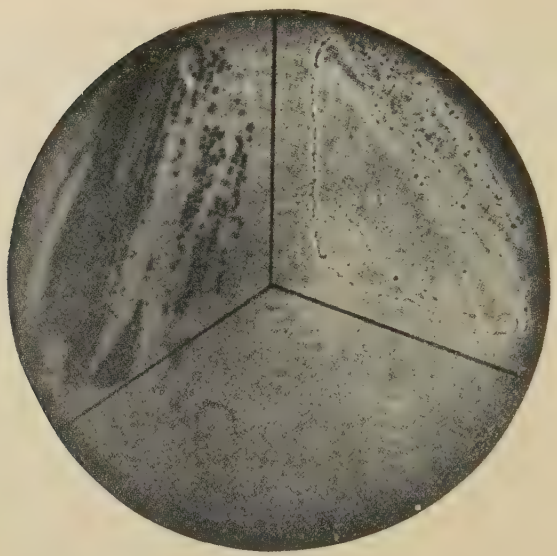


Fig. 88.—Plain blood-agar plate. The lateral sectors show growth of pneumococcus and Streptococcus hæmolyticus. The lower sector shows H. influenzæ; growth is present, but not visible in the photograph (Pritchett and Stillman, Jour. Exper. Med.).

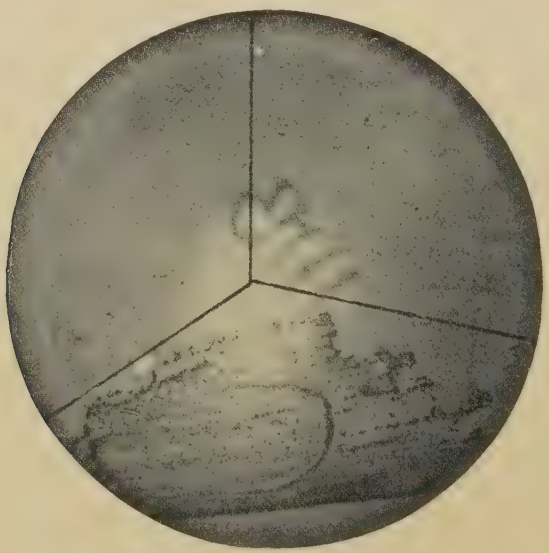


Fig. 89.—Oleate hemoglobin agar plate. Corresponding sectors are planted with the same organisms as in Fig. 88 and show enhanced growth of B. influenzæ in the lower sector and complete inhibition of growth of pneumococcus and Streptococcus hæmolyticus (Pritchett and Stillman, Jour. Exper. Med.).

This medium is well adapted for keeping stock cultures of the Pfeiffer bacillus and for obtaining heavy growths for agglutination and other purposes, but it does not differentiate and is not especially suitable for primary isolation.

Biochemical Reactions.—Fifty per cent. or more of the strains of the Pfeiffer bacillus that have been tested produce indol. This character when present serves to distinguish H. influenzæ from H. pertussis, indol formation never having been observed in the latter organism.[1] Indol production has not so far been definitely correlated with other biological characters. All strains of Pfeiffer bacilli tested by the writer reduce nitrate to nitrite, a further differential character, since H. pertussis and H. bronchisepticus do not possess this quality.[1] Fermentation of dextrose and other carbohydrates, occasionally with gas production,[2] is said to be a characteristic of certain strains.[3] Rivers[4] has shown that some strains produce amylase. In a study of hemophilic bacilli from normal mouths Pritchett and Stillman[5] found that some strains were able to hemolyze blood. The hemolytic strains of H. influenzæ do not appear to be clearly marked off from the non-hemolytic strains by any other differential characters, since indol production and carbohydrate fermentation occur in both groups. Further study may perhaps give a basis for establishing a subgroup of the hemolytic and non-hemolytic groups of H. influenzæ. (Cf. Stillman and Bourn.[3]) At present well-marked subdivisions of H. influenzæ can hardly be recognized.

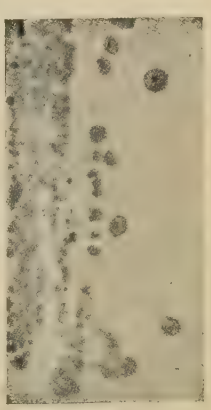


Fig. 90.—Pure culture of H. influenzæ on oleate hemoglobin agar showing large nucleated colonies after thirty-six hours' incubation (Pritchett and Stillman, Jour. Exper. Med.).

Toxic substances are produced in fluid cultures, are filterable, and may appear in appreciable quantities after six to eight hours' incubation. Relatively large quantities of the filtrate (2 to 4 c.c.)

[1] Jordan: Amer. Jour. of Pub. Health, 1920, 10, p. 648.
[2] Wadsworth and Wheeler: Unpublished observations.
[3] Stillman and Bourn: Jour. Exper. Med., 1920, 32, p. 665.
[4] Rivers: Bull. Johns Hopkins Hosp., 1920, 31, p. 50.
[5] Pritchett and Stillman: Jour. Exper. Med., 1919, 29, p. 259.

are necessary to produce death in rabbits.[1] The nature of the toxic substances is uncertain. It is possible that a true toxin is formed,[2] but there is evidence against this view.[3]

Toward external conditions the Pfeiffer bacillus shows little resistance. Desiccation is quickly fatal: a pure culture suspended in water and then dried on silk threads loses its vitality within twenty-four hours; in dried sputum life is maintained somewhat longer, but not, as a rule, beyond forty-eight hours. The bacilli are readily killed by disinfectants.

Effect on Animals.—Animal inoculations with the Pfeiffer bacillus carried out by Pfeiffer and others did not succeed in reproducing the picture of influenza and often had little or no pathologic effect. The pathogenicity of some strains for the white mouse, the guinea-pig, and the rabbit is, however, undoubted. The white mouse seems to be especially susceptible.[4]

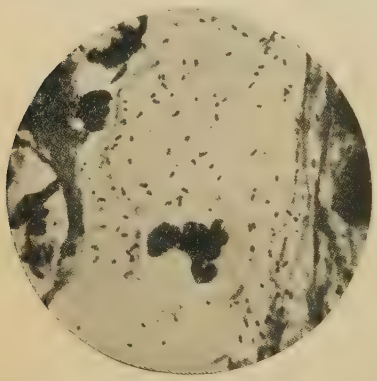


Fig. 91.—H. influenzæ in sputum. Fuchsin stain. Beck prep. (Kolle and Wassermann).

Highly interesting results have been obtained by Blake and Cecil,[5] who by inoculation of the mucous membrane of the upper respiratory tract of normal monkeys succeeded in producing "an acute respiratory disease resembling influenza." The strain of organism used had its virulence first increased by successive animal passages through a series of 11 white mice followed by a series of 13 monkeys. The increased virulence so obtained is lost with extraordinary rapidity when subcultures are made on artificial media outside the animal body. The disease thus produced in monkeys by virulent cultures of H. influenzæ is considered by these experimenters to be essentially

[1] Parker: Jour. Amer. Med. Assoc., 1919, 72, p. 476; Huntoon and Hannum: Jour. Immunol., 1914, 4, p. 167; Ferry and Houghton: Jour. Immunol., 1919, 4, p. 233.

[2] J. Parker: Jour. Immunol., 1919, 4, p. 331.

[3] M. Wollstein: Jour. Exper. Med., 1919, 30, p. 515.

[4] M. Wollstein: Jour. Exper. Med., 1911, 14, p. 63; Ibid., 1915, 22, p. 445; Ferry and Houghton: Jour. Immunol., 1919, 4, p. 233; Albert and Kelman: Jour. Infect. Dis., 1919, 25, p. 433.

[5] F. G. Blake and R. L. Cecil: Jour. Exper. Med., 1920, 32, p. 691.

identical with influenza with respect to its clinical course, symptoms, and complications. The pathologic changes are also thought to be similar in practically all respects to spontaneous, uncomplicated H. influenzæ pneumonia in man.[1]

Antibodies.—The production of antibodies in animals inoculated with H. influenzæ has been observed by a number of investigators. Agglutinin formation has been most completely studied. The titer reached is not, as a rule, very high, but the extremely low results reported by some observers (*e. g.*, 1 : 80) are probably due to the test methods employed. It is desirable to test the agglutinative power of the immune serum by a relatively long incubation at 55° C. in the water-bath.

With suitable methods it is not difficult to obtain a serum titer of 1 : 1600 for the homologous strain. Other (heterologous) strains are not usually agglutinated to so high a titer, *and do not absorb the specific agglutinins produced by the serum strain.* So far as agglutinin production is concerned, therefore, a large number of immunologically different strains of the Pfeiffer bacillus exist; since identical strains were found but rarely during the 1918–19 pandemic, several different strains being sometimes found at the same time in one individual, this fact is regarded by some as evidence that the pandemic was not due to a distinct, especially virulent strain of the Pfeiffer bacillus.

Complement-fixation by specific antibodies found in immunized rabbits may be demonstrated, but the existence of definite groups among the hemophilic bacilli has not been established by this method.

Pathogenicity for Man.—The pathogenicity of the Pfeiffer bacillus for man is shown among other things by the occurrence of cases of meningitis, mostly in infants and of a high fatality, in which the Pfeiffer bacillus is found in pure culture in the cerebrospinal fluid.[2] These cases of "influenzal meningitis," while not very numerous, show that certain strains of this organism possess a definite invasive power. Occasional cases of otitis media, appendicitis, sinusitis, and other localized infections are apparently sometimes caused by the Pfeiffer bacillus.

[1] Blake and Cecil: Jour. Exper. Med., 1920, 32, p. 719.

[2] Wollstein: Amer. Jour. Dis. Children, 1911, 1, p. 42; D. J. Davis: Ibid., p. 249.

In infections of the respiratory tract the Pfeiffer bacillus is frequently present, and it is found on autopsy in pneumonic lesions under conditions where its destructive action upon the tissues can hardly be doubted. Whether it is present as a primary or secondary invader in these cases is more uncertain. Its common occurrence in diseases like measles, whooping-cough, and tuberculosis indicates that its growth on human tissue is favored by the presence of other infecting agents. It seems probable that in respiratory infections the Peiffer bacillus commonly follows in the wake of some other organism.

Inoculation experiments upon man with pure cultures of the Pfeiffer bacillus have given a surprisingly large number of negative results.[1] Enormous numbers of Pfeiffer bacilli instilled into the nose, eyes, and throat have failed to cause any illness. On the other hand, the case reported by D. J. Davis[2] indicates that certain definite symptoms—albeit not those of true influenza!—may follow the inoculation of the throat with pure cultures of this organism.

The Koch-Weeks Bacillus.—A small bacillus, first observed by Koch[3] in 1883 in a series of eye inflammations in Egypt, was successfully cultivated by Weeks[4] in New York in 1890, and is now recognized as the cause of a world-wide and highly contagious form of conjunctivitis (Fig. 92). Several recent investigators believe the Koch-Weeks bacillus and the Pfeiffer bacillus to be identical and there is much evidence to support this view.[5] They are alike also in their staining reaction.

Growth may occur on ordinary nutrient agar at 37° C., but most success is obtained with serum-agar, or a mixture of glycerol-agar and ascitic fluid (2 : 1). The colonies appear as minute, projecting, transparent dots, which tend to become confluent; they never attain a large size and are easily detached from the medium. The bacilli possess slight powers of resistance, and there is little reason to suppose that dust is a common means of conveying infection. Direct contact with infective material through the medium of hands, towels, handkerchiefs, etc., must rather be considered the

[1] Rosenau: Jour. Amer. Med. Assoc., 1919, 73, p. 311; McCoy and Richey: Pub. Health Repts., 1919, 34, p. 33.

[2] D. J. Davis: Jour. Infect. Dis., 1906, 3, p. 1.

[3] Koch: Arb. a. d. k. Gesund., 1887, 3, Anlagen, p. 62.

[4] Weeks: N. Y. Med. Rec., 1887, 31, p. 571.

[5] Knohr: Deut. med. Wchnschr., 1925, 51, p. 65.

usual mode of conveyance. Flies may also be concerned in transmission. A peculiar form of hand infection due to an organism probably identical with the Koch-Weeks bacillus has been described by McDill and Wherry.[1]

Whooping-cough.—Whooping-cough is one of the serious diseases of childhood. In 1925 in the "registration area" of the United States, comprising about 90 per cent. of the population of the country, there were reported 6,948 deaths from this cause. This amounted to a death-rate more than double that for the much more dreaded scarlet fever. Many deaths among children reported as due to bronchopneumonia are also, in reality, attributable to whooping-cough. Ninety-six per cent. of the deaths from whooping cough are in children under five years of age.

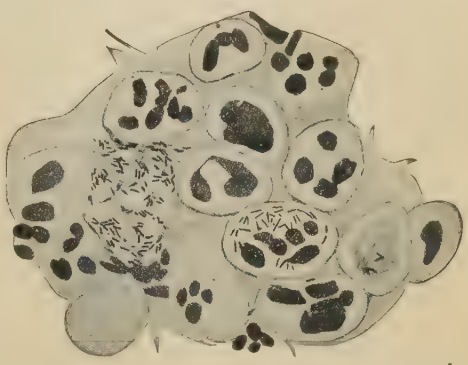


Fig. 92.—Koch-Weeks bacillus in conjunctivitis; × 900 (Axenfeld, Kolle and Wassermann).

Bacilli resembling Bacillus influenzæ were reported by early observers as occurring in a large proportion of cases of whooping-cough. Although there are minor differences in the descriptions of these organisms as given by different observers, the cultural and morphologic characters are essentially similar, and there seems little doubt that Spengler, Jochmann and Krause, Wollstein, and Davis[2] had the same bacillus in hand. Owing to this organism's close resemblance to, if not identity with, the influenza bacillus, there was considerable reluctance among bacteriologists to accept it as the cause of whooping-cough.

Bordet and Gengou[3] found a characteristic short oval bacillus, B. pertussis, in the bronchial exudate from cases of whooping-cough. It is present in great numbers in the early stages of the disease. It grows feebly on a special medium devised by Bordet and Gengou,

[1] McDill and Wherry: Jour. Infect. Dis., 1904, 1, p. 58.
[2] Davis: Jour. Infect. Dis., 1906, 3, p. 1.
[3] Bordet and Gengou: Ann. de l'Inst. Past., 1906, 20, p. 731.

consisting of 1 per cent. glycerol-agar or glycerol-broth made with macerated potato and added to an equal volume of human or rabbit blood. Later workers have been unable to obtain initial growth except upon this medium. After some generations on the potato-blood-agar mixture it will grow on the surface of ascitic-fluid agar in a whitish, elevated streak which does not spread far over the surface. It can also be grown on veal agar and in veal broth. It is non-motile, gram-negative, and shows polymorphism in fluid media. In size it averages slightly larger than the influenza bacillus. The single colonies on solid media may reach a diameter of 2 to 4 millimeters in forty-eight hours. A little later they acquire a slight brownish color. Mucoid substance is abundantly produced by the culture and the growth is sticky and tenacious. B. pertussis is agglutinated by the serum of convalescents from whooping-cough, although with great inconstancy. The serum of convalescents also shows the presence of a specific substance by giving the complement-fixation reaction (p. 177). This property is constant. The bacillus has a distinct toxic action upon the tissues of the guinea-pig and rabbit.

Klimenko[1] found the Bordet-Gengou bacillus in 80 per cent. of the children (76 in number) that he examined during the first week of the disease. He also succeeded in reproducing the disease with pure cultures in 48 young dogs and in monkeys.

Cultures from carefully washed sputum or obtained by the cough plate method have been advocated as a means of diagnosis of the disease in its early stages.[2] After the fifth week only a small percentage of positive cultures is found (4.0) as compared with the percentage during the catarrhal period (59.0) or in the first week of the whoop (53.0). It is believed that carriers, secondary cases and clinically doubtful cases may be detected by this means, and that eventually release from quarantine may be based on bacteriological examination rather than on arbitrary time limit. Whooping cough stations for diagnosis have been established, it is said with great success, in Copenhagen.

Pathogenicity.—Important work upon the relation of B. pertussis to the characteristic manifestations of whooping-cough has

[1] Klimenko: Centralbl. f. Bakt., I, Orig., 1909, 48, p. 64.

[2] Madsen: Boston Med. and Surg. Jour., 1924, 192, p. 50. Lawson and Mueller: Jour. Am. Med. Assn., 1927, 89, p. 275.

been carried out by Mallory and his co-workers.[1] The production of a mild toxin by the bacillus and the absorption of the toxin seem to be shown by the exudation of leukocytes into the lumen of the trachea and bronchi, by slight changes in the lymph-nodules of the spleen, lymph-nodes, and gastro-intestinal tract, by the occurrence of the well-known lymphocytosis of whooping-cough, and by the production of the antibody which makes possible the specific complement-fixation reaction. Possibly the cilia are damaged by the toxin, but this is not certain. More important than toxic action seems to be the mechanical disturbance caused by the presence of the bacilli in the respiratory tract. By their presence in enormous numbers, "dozens to a hundred or more between the cilia of a single cell," they are thought to interfere seriously with the normal ciliary action (Fig. 93). In consequence, the removal of secretions and of inhaled particles is prevented, and the lungs are probably more exposed to infection by inhalation than under ordinary circumstances. The bronchopneumonia which sometimes develops in fatal cases of whooping-cough may be due to B. pertussis or to other organisms, such as the pneumococcus.

Fig. 93.—Whooping-cough. Minute bacilli present in masses between cilia of two cells lining the trachea; × about 1500 (Mallory and Horner).

Mallory has also observed the characteristic lesions of whooping-cough in young animals (puppies and rabbits) after inoculation with whooping-cough sputum and with pure cultures of the Bordet-Gengou bacillus. From these lesions B. pertussis has been again isolated in pure culture.

Nicolle and Conor[2] have reported favorable results in the amelioration of symptoms following the injection of a vaccine made of living cultures of the Bordet-Gengou bacillus. Sill[3] has also obtained good results both in treatment and in prophylaxis. "Thirty-three cases of whooping-cough were treated with the pertussis vaccine, and in all the effect of the vaccine was to diminish

[1] Mallory et al.: Jour. Med. Res., 1912–13, 22, pp. 115, 391.
[2] Nicolle and Conor: Ref . Jour. Amer. Med. Assoc., 1913, 61, p. 209.
[2] Sill: Amer. Jour. Dis. Children, 1913, 5, p. 379.

markedly the number and severity of the paroxysms and the amount of vomiting." As yet, however, the results, both curative and preventive, with pertussis vaccine are far from being generally convincing. Controlled observations on a large series of cases are needed before judgment can be given.

CHAPTER XXI

THE SPORE-FORMING ANAËROBES

Genus: Clostridium. Anaërobes or micro-aërophiles. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

Type species is Clostridium butyricum, Prazmowski.

The important pathogenic organisms that fall in this group are all rather large-sized rods, forming resistant spores and needing a low oxygen tension (anaërobic conditions) for their successful cultivation. Renewed interest in the sporulating anaërobes has been excited by the prominence in the Great War of anaërobic wound infections, and by the apparent increase of botulism in the United States. Tetanus or lockjaw, however, remains the best understood disease due to an obligate anaërobe.

Tetanus

Tetanus is a disease of man and animals characterized by spasms of the voluntary muscles. The spasms are often most marked in the muscles of the jaw and neck, hence the name lockjaw. The tetanus bacillus was first described in 1884 by Nicolaier,[1] who observed it in the pus taken from mice and other animals that had died after subcutaneous inoculation with small quantities of soil. He failed to secure pure cultures, but Kitasato[2] succeeded in doing so by the application of special methods of culture, and by making use of the high resistance of the spore to eliminate the associated non-sporulating aërobes. With such cultures he demonstrated Koch's postulates for tetanus. He further proved the inability of the tetanus bacillus to invade the blood-stream and showed the disease to be an intoxication.[3] In 1890 von Behring and Kitasato[4] laid the basis for antitoxic therapy in their discovery of diphtheria

[1] Nicolaier: Deut. med. Wchnschr., 1884, 10, p. 842.
[2] Kitasato: Ztschr. f. Hyg., 1889, 7, p. 225.
[3] Kitasato: Ztschr. f. Hyg., 1891, 10, p. 267.
[4] Behring and Kitasato: Deut. med. Wchnschr., 1890, 16, p. 1113.

and tetanus antitoxin. Recent investigations of Tulloch[1] have differentiated by the agglutination reaction four serologic types of Cl. tetani.

Clostridium tetani is widely distributed in nature. The literature on its presence in fertilized soil, street dust, hay dust, mud, fresh and salt water, bilge-water of ships, gun wads, wearing apparel, and the feces of men and animals, notably horses, has been fully reviewed by Noble.[2] Tetanus bacilli have been recovered from court plaster by McCoy, Leake and Corbitt[3] and from gelatin by Smith,[4] who emphasized the danger of their presence in vaccines and serums used parenterally.

Fig. 94.—Clostridium tetani showing spores. Pure culture on agar. Fuchsin stain (Kolle and Wassermann).

It is an open question to what extent the organism multiplies outside the animal body. Sormani (see Noble[2]) has held that the principal natural habitat of Cl. tetani is the intestinal tract of vertebrates, and that the toxic properties of soil, water, gelatin, glue, etc., are attributable mainly to persistent and resistant spores of fecal origin. Certain horses may be "carriers" of these spores for at least several months, and Tenbroeck and Bauer[5] found that 34.7 per cent. of a group of Chinese men in Peking had tetanus bacilli in their stools.

Individual tetanus bacilli are slender, motile, peritrichally flagellated (20 to 30), gram-positive, sporulating rods with rounded ends (Fig. 94). Their common dimensions are 0.3 to 0.5 μ by 2 to 5 μ, but vegetative filaments of much greater length occur. The shorter forms are usually straight; filaments tend to curve in an undulating manner. Short chains of rods occur.

[1] Tulloch: Jour. Roy. Army Med. Corps, 1917, 29, p. 631 (Abst. in Bull. de l'Inst. Past., 1918, 16, p. 431); Proc. Roy. Soc., London, Ser. B, 1918, 90, pp. 145, 529; Jour. Hygiene, 1919, 18, p. 103.

[2] Noble: Jour. Infect. Dis., 1915, 16, p. 132.

[3] McCoy, Leake, and Corbitt: Public Health Reports, U. S. P. H. S., 1917, 32, p. 1450.

[4] Smith, Theobald: Jour. Amer. Med. Assoc., 1908, 50, p. 929.

[5] Tenbroeck and Bauer: Jour. Exper. Med., 1922, 36, p. 261.

Cultural Characteristics.—Cl. tetani is an obligate anaërobe. It develops readily in plain broth, brain, meat, agar, and gelatin media from which the air has been expelled by heating and excluded by some form of seal. If the depth of medium is adequate, say 10 to 12 cm., no special seal is required, especially for the more viscous media. For liquid media, some form of mechanical seal is desirable, such as the marble seal in the constricted tube of Hall.[1] This device, using meat infusion peptone broth without sugar, is recommended for initial cultures from which pure cultures can be isolated readily by deep agar or surface culture methods (Hall[2]). Deep meat or brain media are also suitable for primary culture.

It is difficult for inexperienced workers to isolate the tetanus bacillus in pure culture. The inoculation of suspected material into mice, rats, or guinea-pigs was formerly considered a desirable preliminary step. But Bengtson and McCoy[3] were able by direct inoculation to detect tetanus bacilli in only three out of thirty-five samples of artificially seeded smallpox vaccine virus, all of which were positive when cultures were injected. Heating soil, pus, or mixed cultures to 80° C. for one-half hour (Kitasato) has been supposed to simplify isolation by destroying the vegetative forms of other organisms that are present; but this method may leave the spores of the tetanus bacillus still badly mixed with those of other aërobic and anaërobic bacteria. Many of these are more resistant to heat than those of Cl. tetani; in such cases selective dyes may be used to advantage (Hall[4]).

The growth of Cl. tetani is influenced greatly by the presence of associated microörganisms. In sugar-free media Cl. tetani may grow in mixed cultures upon the surface of culture-media in contact with air through the absorption of oxygen by the associated aërobes. But in glucose broth the growth of Cl. tetani in mixed culture is likely to be inhibited by acid formation due to the associated organisms. Sugar-free media are therefore preferable for initial culture from contaminated material.

In pure culture, glucose stimulates growth as in the case of other anaërobes, although glucose and other carbohydrates are not hydrolysed by Cl. tetani. But sporulation is not inhibited by

[1] Hall: Univ. of Calif. Pub. in Pathology, 1915, 2, p. 147.
[2] Hall: Jour. Infect. Dis., 1920, 27, p. 576.
[3] Bengtson and McCoy: Amer. Jour. Pub. Health, 1919, 9, p. 427.
[4] Hall: Jour. Amer. Med. Assoc., 1919, 72, p. 275.

carbohydrates as with the fermentative anaërobes; on the contrary, sporulation of Cl. tetani is accelerated in glucose broth.

The spores of Cl. tetani are highly resistant, Henrijean[1] having kept them alive, protected from light and heat, for eleven years. Smith found a number of strains that resisted steaming at 100° C. for forty to sixty minutes. They are correspondingly more resistant to disinfectants than non-sporulating bacteria. Five per cent. phenol is said to destroy them in ten to twelve hours; the addition of 0.5 per cent. hydrochloric acid may reduce the time to two hours. Mercuric bichlorid 1:1000 kills in two to three hours, with 0.5 per cent. hydrochloric acid in thirty minutes. One per cent. silver nitrate kills in one minute.

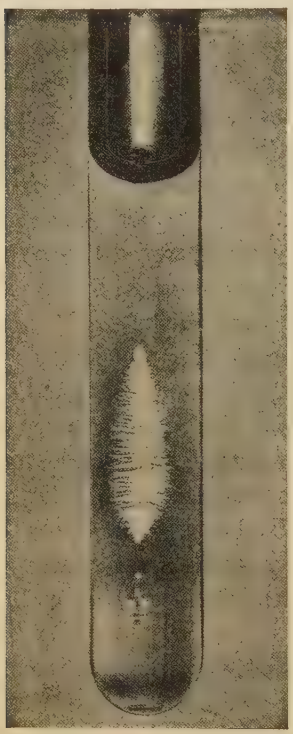


Fig. 95.—Clostridium tetani, gelatin stab-culture, six days (Fränkel and Pfeiffer).

The minimum temperature for growth is 14° C., the optimum 37° C., and the maximum 43° C. Spore formation begins in about two days at 37° C., and in eight to ten days at room temperature.

Growth in either plain or glucose broth produces moderate turbidity with evolution of a very slight amount of gas and an alkaline reaction. Isolated colonies in deep glucose agar are usually woolly and may be either flocculent or have an opaque center. A deep agar stab-culture resembles an inverted fir tree with delicate twigs and branches (Fig. 95). Growth in gelatin is slow at room temperature, the colonies first being visible in about three days; under a low power of the microscope filaments may be observed radiating from a central core; the gelatin is slowly liquefied. Gelatin cultures incubated at 37° C. for twenty-four to forty-eight hours usually fail to stiffen in the ice-chest. Coagulated protein such as blood-serum and egg white, are very slowly liquefied. Deep brain and meat media may be slightly softened, but never digested fully; after several weeks a slight darkening occurs near the surface

[1] Henrijean: Ann. de la soc. mèd. chir. de Liège, 1891.

exposed to the air. There may even be a slight but distinct black discoloration which is intensified by metallic iron or certain iron salts in these media.

None of the following carbohydrates, glucosids, or alcohols is fermented by Cl. tetani: glucose, levulose, galactose, maltose, saccharose, lactose, inulin, starch, mannit, dulcit, glycerol, salicin. Statements to the contrary in various text-books are based probably upon the study of cultures containing other anaërobes.

Surface growth may be secured on slanted plain or blood-agar by the method of using alkaline-pyrogallol in the cotton plug.[1] Colonies appear in twenty-four to forty-eight hours; they are flat, rhizoid, or even feathery, and frequently exceed 1 mm. in diameter. Later the centers may become slightly raised. Blood-cultures show hemolysis.

The products of growth include a small amount of gas which is derived from the protein metabolism, since the addition of sugars does not increase its volume. In gelatin and brain media gas is produced more abundantly than in ordinary peptone media. The composition of the gas is unknown. The gas has a characteristic odor unlike that of most other bacteria, aërobic or anaërobic. It certainly contains sulfuretted hydrogen, as can be shown by the blackening of lead acetate paper. Indol is also produced abundantly. But the most important metabolic product of growth *in vitro* or *in vivo* is the powerful toxin of the tetanus bacillus.

Tetanus Toxin.—Liquid cultures of Cl. tetani are usually highly toxic; 0.000,005 c.c. or less may be fatal to a mouse weighing 10 grams. Hall[2] repeatedly produced toxin of which the M. L. D. for guinea-pigs was rarely more than .0001 c.c. and usually less. Ruediger[3] was equally successful, and in one instance produced tetanus toxin of such strength that 1 c.c. would have sufficed to kill 50,000 guinea-pigs.

Toxic cultures may be freed from bacteria by filtration through filters of the Berkefeld, Pukal, Nordmeyer, or Mandler type. A preservative such as tricresol or phenol, already diluted 1 to 10 in sterile distilled water, may be added before or after filtration, in a proportion of 1 part diluted antiseptic to 30 parts of toxin—thus

[1] Kürsteiner. Centralbl. f. Bacteriol., II, 1907, 19, p. 99.
[2] Hall: Univ. of Calif. Pub. in Pathology, 1913, 2, p. 97.
[3] Ruediger: Philippine Jour. of Sci., 1915, 10, p. 31.

giving a final concentration of 0.33 per cent. The filtered toxin may be preserved in tightly stoppered brown bottles in a dark, cold place, since it is highly unstable and is destroyed in aqueous solution by exposure to light, heat, and chemical action. Kitasato found that exposure of toxic broth filtrates to 55° C. for one and a half hours, 60° C. for twenty minutes, or 65° C. for five minutes reduced the potency considerably. Tetanus toxin is destroyed also by gastric and tryptic digestion. It is the general practice in immunizing horses for the production of antitoxin to use the toxic filtrate in as fresh a state as possible.

Before the toxin can be so used it must be tested for potency. But the minimum lethal dose need not be determined with great exactness; if 1 c.c. of a 1 : 10,000 dilution kills a guinea-pig with tetanus symptoms, the filtrate may be accepted. The New York Board of Health generally uses two animals in a test, one inoculated with 1 c.c. of 1 : 15,000 dilution and one with 1 c.c. of 1 : 25,000. Tests of higher dilution are rarely required except for research purposes. Animal inoculation is the only means of determining the toxicity of tetanus culture filtrates; while filtrates are often gelatinolytic, the gelatinase is distinct from the toxin.[1]

Brieger and Cohen[2] precipitated the toxin from a broth culture filtrate with ammonium sulfate and secured it as a dry powder free from phosphorus and nearly free from sulfur. It was fatally toxic for white mice in a dose of 0.000,000,05 gram. London and Aristovsky[3] prepared tetanus toxin of even higher potency, 0.000,000,02 gram being fatal for white mice in two days. No one pretends to have obtained tetanus toxin in a pure state.

The amount of toxin required to kill different animals varies considerably, as the following comparative figures from Knorr[4] show:

1 gram of horse is destroyed by			x toxin.
1 gram of goat	"	"	2x "
1 gram of mouse	"	"	13x "
1 gram of rabbit	"	"	2,000x "
1 gram of hen	"	"	200,000x "

Cold-blooded animals are not susceptible to tetanus toxin.

[1] Fermi and Pernossi: Centralbl. f. Bakt., 1894, 15, p. 303.
[2] Brieger and Cohen: Ztschr. f. Hyg., 1893, 15, p. 8.
[3] London and Aristovsky: Comptes rend. Soc. biol., 1917, 80, p. 756.
[4] Knorr: Münch. med. Wchnschr., 1898, p. 321.

An incubation period is always observed, and this cannot be shortened beyond a certain minimal limit, (*e. g* ., in the mouse eight hours), even with large doses. Otherwise the incubation period and the time of death are inversely proportional to the amount of toxin injected.

While natural tetanus is usually the result of the subcutaneous introduction of tetanus spores simultaneously with foreign matter, including saprophytic and other pathogenic bacteria which may lead to suppuration or even gaseous gangrene, the local reaction is often insignificant and visible changes in the tissues are not marked. The specific bacillus multiplies locally, but does not spread throughout the body. The symptoms are attributable mainly to a disturbance of the central nervous system, as evinced in the characteristic muscular spasms, and depend upon the absorption of the toxic products of the bacilli.

Meyer and Ransom[1] showed that tetanus toxin is absorbed by the end organs of the motor nerves only, and travels to the ganglion cells of the central nervous system not by way of the blood or lymph-channels, but along the axis-cylinders of the peripheral nerves. The time consumed in this passage represents the larger part of the incubation period. The toxin may circulate for a time in the blood, but the only path to the central nervous system lies along the axis-cylinders of the motor nerve tract. Intravenous injection of toxin into experimental animals causes only general tetanus, according to Ransom's recent reiteration of this view.[2] A cut nerve takes up the toxin very slowly and a degenerate nerve not at all. Section of the spinal cord prevents the toxin from reaching the brain. Meyer and Ransom believed that the spinal ganglion of the sensory nerve presents a barrier to the advance of the toxin, and that for this reason sensory nerves are unable to conduct the toxin. The remarkable excitation of the motor cells of the spinal cord that is observed in tetanus is unaccompanied by characteristic lesions.

Tetanus toxin possesses a strong affinity for the cells of the central nervous system, as evidenced by the now classic experiments of Wassermann and Takaki.[3] A mixture of tetanus toxin and brain substance can be injected into an animal without produc-

[1] Meyer and Ransom: Archiv. f. Exper. Path. u. Pharm., 1903, 49, p. 369.
[2] Ransom: Lancet, 1917, 2 [193], p. 928.
[3] Wassermann and Takaki: Berl. klin. Wchnschr., 1898, 35, p. 5.

ing any toxic effect, the toxin apparently entering into so firm a combination with some ingredient of the nervous matter that it is powerless to affect the living organism. As pointed out elsewhere (p. 174) this fact is regarded as strongly supporting the receptor theory of antitoxin production. Not only the central nerve-cells, but to some extent other tissue cells, are able to bind tetanus toxin. Subcutaneous inoculation is less likely to result fatally than direct inoculation into nerve tissues, for the reason that in the former case the cells of the liver, kidney, connective tissues, etc., anchor the tetanus toxin and prevent it from reaching the highly sensitive nerve-cells. The exquisite susceptibility of an animal like the guinea-pig to tetanus toxin is perhaps correlated with the inability of the non-nervous tissues to bind the poison, thus leaving the toxin free to make its way to the central nervous system.

The muscular cramps which characterize tetanus are due to a particular substance, the so-called *tetanospasmin*. It is this poison which has such strong affinity for the nervous system of susceptible animals. The existence of a second toxin, *tetanolysin*, was first shown by Ehrlich. Tetanolysin, which is probably of less importance than tetanospasmin, exhibits special affinity for the red blood-corpuscles, with which it unites, producing laking. The two toxic bodies are quite distinct as regards combining relations and other properties, each giving rise to its specific antitoxin. It is not yet certain whether tetanolysin has any significance in connection with the ordinary symptom-complex.

Pathogenicity for Man.—An excellent presentation of this subject will be found in the article, "Tetanus," by C. H. Browning.[1] Tetanus is distinctly an intoxication dependent upon the development of infection in a deep, dirty wound. Such a wound may be relatively small. The wide-spread occurrence of the tetanus bacillus seems at first glance out of harmony with the relative infrequency of tetanus infection, but mere introduction of the bacillus into the body is not sufficient to produce the disease. The organisms must find favorable conditions for proliferation at the site of their penetration and lodgment. Experimently, pure cultures of tetanus rods or spores that have been freed from toxin cannot germinate in the animal body when inoculated in moderate numbers, and are hence innocuous. But simultaneous inoculation

[1] Browning: Brit. Jour. Surgery, 1916, 4, p. 14.

with common saprophytes, such as B. prodigiosus, or with certain chemical substances, such as lactic acid, enables the bacilli to proliferate and form their toxin.

Common experience has taught that tetanus develops most frequently in connection with punctured or contused wounds, and especially when the tissues suffer considerable injury and much foreign matter containing tetanus bacilli is forced far into the wound. Conditions favorable to the development of tetanus occur in war wounds, in injuries to stablemen and others whose occupation brings them into frequent contact with the dung of animals, in injuries due to firecrackers and toy pistols, in septic midwifery, after hemostatic injection of gelatin, and, rarely, after the prophylactic and therapeutic use of biologic products.

In the early days of the Great War tetanus took a tremendous toll. Sanford,[1] who reviewed the literature of tetanus in the war, gives the incidence among the wounded men of the British forces as 1.6 per cent. in September, 1914, and 3.2 per cent. in October. The statistics for the French and German troops were unreliable, but previous to the general use of antitoxin the incidence of tetanus is known to have been high.

The high incidence in the first months of the Great War is probably connected with the highly fertilized character of the soil upon which the battles were fought, the intimate contact of the soldiers and their clothing with the soil in trench life, and the extensive use of high explosives in trench mortars and hand grenades. Wounds from bombs that exploded after striking the ground were much more prolific of tetanus and other anaërobic infections, such as gaseous gangrene, than rifle-bullet wounds. After the first two months of the war the prophylactic use of tetanus antitoxin in soldiers with lacerated wounds became wide-spread and effected a notable decrease in tetanus morbidity, as Cummings and Gibson[2] and Bruce[3] showed. The recognition of trench foot as a form of wound favorable to the development of tetanus also led to a reduction in mortality through the timely prophylactic use of tetanus antitoxin (from 75 per cent. before 1917 to 16.6 per cent. thereafter).

[1] Sanford: Internat. Assoc. of Med. Museums, Bull. No. 7, 1918, p. 365.
[2] Cummings and Gibson: Lancet, 1919, 196, p. 325.
[3] Bruce: Jour. of Hygiene, 1920, 19, p. 1.

A high incidence of tetanus following Fourth of July celebrations in the first years of the 20th century (cf. p. 397) was assumed to be due to infection from the material entering into the composition of the explosives; but Wells[1] failed to find Cl. tetani in 200 blank cartridges from five firms.

Hill[2] has stated that in the Canal Zone about 86 per cent. of the cases of tetanus occurred in children under one year of age and tetanus neonatorum was especially prevalent. In tetanus neonatorum septic midwifery is responsible for infection of the umbilicus, and this form of tetanus is especially rife among the negroes of the Southern States and in other races living under unhygienic conditions. Anders and Morgan[3] collected statistics covering 4878 cases of tetanus neonatorum that occurred from 1870 to 1900. There is one famous case of a small island near Iceland with a total population of about 200 in which 185 newborn children perished from tetanus during twenty-five years. Foreign investigators have recorded a few cases of tetanus following the hemostatic injection of unsterilized gelatin.

Before the necessity of rigid tests of biologic products intended for parenteral prophylaxis or therapy was appreciated several unfortunate outbreaks of tetanus occurred owing to contamination with tetanus spores or toxin. Thus a quantity of diphtheria antitoxin derived from a horse in the presymptomatic stage of tetanus was distributed in St. Louis before suitable tests were made, and the administration of this serum to diphtheritic patients was followed by fatal tetanus in a number of cases. Although the horse from which the serum was drawn showed no symptoms at the time of bleeding, it later developed tetanus and died. The amount of toxin in the serum was so great that 0.1 c.c. killed a guinea-pig in a few days with tetanus, and nearly all of the children who received as much as 10 c.c. died.[4]

An unfortunate outbreak of tetanus occurred in 1902 at Mulkowal in British India during the course of a series of inoculations of Haffkine's plague prophylactic. In a total of 107 persons given the prophylactic, 19 developed symptoms of tetanus and died. The present requirements of the United States Public Health

[1] Wells: Phila. Med. Jour., 1900, 5, p. 1377.

[2] Hill: Arch. of Int. Med., 1911, 8, p. 747.

[3] Anders and Morgan: Jour. Amer. Med. Assoc., 1906, 47, p. 2083.

[4] Bolton and Fisch: Trans. Assoc. Amer. Phys., 1902, 17, p. 462.

Service for sterility and safety (animal) tests of biologic products which are intended for parenteral administration and are manufactured by concerns engaged in interstate commerce, are framed to preclude the recurrence of such deplorable accidents.

There is strong evidence that the cases of tetanus occasionally observed following vaccination against small pox are rarely due to contamination in the vaccine virus. Francis[1] reported that in twelve years no commercial smallpox vaccine has been found naturally contaminated with tetanus. Moreover, it is difficult, if not impossible, to produce tetanus in laboratory animals with vaccine containing large numbers of tetanus organisms purposely placed therein. In addition, Anderson[2] has pointed out that, while the average period from vaccination to onset of symptoms (83 cases) was over twenty days, the average mortality was slightly higher than the mortality of cases of tetanus due to other causes and having an incubation period of ten days or less. Remembering the dictum of Hippocrates that "such persons as are seized with tetanus die within four days, or if they pass these they recover," and the comment of Hill,[3] that "a lapse of twenty-four hundred years and all our modern methods and advanced technic but little modify this statement," it seems likely that cases of tetanus occurring fifteen to twenty days subsequent to vaccination have not received their infection through the vaccine virus. As Geiger[4] and others have illustrated, ample opportunities for subsequent infection at the site of vaccination may occur.

Pathogenicity for Other Animals.—In the horse tetanus is not a rare affection, the symptoms and course of the disease being similar to the disease in man. Cattle, sheep, and hogs are less commonly affected. Experimentally, tetanus can be produced in mice and in guinea-pigs by inoculation of spores introduced upon splinters of wood, and also by injection of toxin. The feeding of animals with tetanus bacilli, spores, or toxin is without effect. Tetanus differs from most infectious diseases in that the diseased animal is not an appreciable factor in the spread of infection. A normal horse apparently may distribute tetanus germs quite as widely and freely as a horse sick with tetanus.

[1] Francis: U. S. Pub. Health Serv. Hyg. Lab. Bull., No. 95, 1914.

[2] Anderson: U. S. Pub. Health Serv. Reprint, No. 289, Pub. Health Reports, 1915.

[3] Hill: Arch. Int. Med., 1911, 8, p. 747.

[4] Geiger: Calif. State Jour. Med., 1917, 15, p. 152.

Immunity.—Man may acquire immunity to tetanus as indicated in the recent observations of Tenbroeck and Bauer,[1] that while a large number of the inhabitants of certain regions in China discharge tetanus bacilli in their stools, tetanus seems to be no more prevalent there than in Europe, for those who harbor tetanus bacilli in their intestinal tracts also have appreciable amounts of tetanus antitoxin or tetanus agglutinins in their blood. Artificial immunity to tetanus depends upon the presence in the body of antitoxin which is able to neutralize the toxin. Antitoxin for therapeutic purposes is produced in healthy horses by active immunization through the repeated subcutaneous injection of increasing doses of toxic filtrates from broth cultures of Cl. tetani. The serum is standardized according to the method of Rosenau and Anderson,[2] in which the American immunity unit was legally defined in 1907 as "ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea-pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service." Thus the tetanus antitoxic unit has slightly more than ten times the experimental protective power possessed by the diphtheria antitoxic unit.

An antitoxic unit strength of 900 per cubic centimeter may be attained exceptionally. Tetanus antitoxin is moderately stable, but should be kept cool and in the dark.

The mode of action of tetanus antitoxin, so far as it is understood, is precisely like that of diphtheria antitoxin. The tetanus antitoxin circulates in the blood and is not taken up by either the central nervous system or the peripheral nerves. It can consequently bind only the toxin that is itself in the circulation. Its curative value is much less than that of the diphtheria antitoxin, partly, perhaps, because the affinity of the tetanus toxin for nerve tissue is stronger than its affinity for the antitoxin, partly, perhaps, because of a relatively slight power of recuperation on the part of the central nervous system, and partly also because the toxin, when once incorporated in the axis-cylinders, is better protected from contact with the antitoxin than if it were in the circulation. According to Behring, there is no hope of success from subcutaneous injec-

[1] Tenbroeck and Bauer: Jour. Exper. Med., 1922, 36, p. 261, and 1922, 37, p. 379.

[2] Rosenau and Anderson: Bull. No. 43, U. S. Pub. Health Serv., 1908.

tion of tetanus antitoxin after symptoms have existed for more than thirty hours. The main value of tetanus antitoxin therefore lies in its prophylactic use.

In veterinary practice, tetanus antitoxin has been used prophylactically with a high degree of success, as the references compiled by MacConkey[1] show. Vaillard collected the statistics of eight veterinary surgeons from 1898 to 1906, who inoculated 13,124 animals after operations or accidental wounds, without the occurrence of a single case of tetanus. During the same time two veterinary surgeons alone saw 139 cases of tetanus among animals which did not receive the treatment. The figures of Nocard and Labat added to Vaillard's data cover the cases of 16,917 animals receiving prophylactic injections; among them one single horse had tetanus. In this case the antitoxin was given five days after the wound and the attack was very benign. The usual prophylactic dose in veterinary practice is 500 units.

It is believed that human tetanus may be prevented almost altogether by the routine administration of sufficiently large doses of antitoxin immediately in all cases of injury of a kind likely to provoke tetanus. The progressive reduction in deaths from tetanus following Fourth of July injuries—from 406 deaths out of 4449 injuries in 1903, to a negligible figure in each of the years 1911 to 1916—may be attributed in part to the increased prophylactic use of antitoxin, although the better and more general cleaning and drainage of such wounds are contributory factors, and the "sane Fourth" agitation has done much to decrease the total of such injuries.[2]

Cases of atypical or chronic tetanus sometimes follow the prophylactic use of antitoxin. This local form of tetanus has been described by Courtois, Suffit, and Giroux.[3]

The curative value of tetanus antitoxin is still doubtful. Previous to the war Ashhurst and Johns,[4] Irons,[5] and Park and Nicoll[6]

[1] MacConkey: Brit. Med. Jour., 1914, 2, p. 609.

[2] Fourteenth Annual Summary of Fourth of July Injuries, Jour. Amer. Med. Assoc., 1916, 67, p. 676.

[3] Courtois, Suffit and Giroux: "Les formes anomales du tétanos," Paris, 1916.

[4] Ashhurst and Johns: Amer. Jour. Med. Sci., 1913, 145, p. 806; 1913, 146, p. 77.

[5] Irons: Jour. Amer. Med. Assoc., 1914, 62, p. 2025.

[6] Park and Nicoll: Jour. Amer. Med. Assoc., 1914, 63, p. 235.

all showed the possibility of reducing the mortality by rational treatment, in which intraspinal injections of antitoxin were a prominent feature. Antitoxin is never the sole reliance in treatment; thorough cleaning and disinfection of the wound are important as well as rest in bed in a dark, quiet room. The following figures on the occurrence and mortality of tetanus among the British troops indicate considerable gain in the efficacy of treatment:

Date.	Cases.	Deaths.	Mortality, Per Cent.
1914	192	104	54.2
1915	134	75	55.9
1916	501	182	36.3
1917	353	68	19.2
1918	266	68	25.5

Yet Cummings and Gibson[1] and Bruce consider that the curative value of tetanus antitoxin has not been strikingly substantiated.

Tetanus antitoxin therapy, nevertheless, offers the only hope of specific cure and should always be used. Various routes of injection are recommended. Animal experiments support the intrathecal method which has the advantage of economizing serum, but most authorities think this should be supplemented with intramuscular and intravenous injections. An intravenous injection of 30,000 units maintains antitoxin in the tissues for thirty-nine days. In the majority of cases this single injection is absolutely sufficient.

Tulloch has shown the existence of four distinct serologic types of tetanus bacillus differentiated by the agglutination reaction. The distribution of these among two separate groups of wounded men, all of whom were inoculated prophylactically with antitoxins, was as follows:

Type.	100 Tetanus Cases.	25 Non-tetanus Cases.
1	41 per cent.	76 per cent.
2	22 "	12 "
3	33 "	8 "
4	4 "	4 "

Tulloch found no experimental evidence to indicate that Type 1 antitoxin lacked protective power against toxins of the other types.

[1] Cummings and Gibson: Lancet, 1919, 1 [196], p. 325.

Gaseous Gangrene

Gaseous gangrene is a syndrome often following dirty, lacerated wounds, especially those involving fractures. Rare in peace times, it is a characteristic complication of war wounds. In its fulminating form the muscles become filled with gas and with a serosanguineous exudate depending for its character upon the associated micro-organisms, for gaseous gangrene is nearly always a mixed infection of aërobes and anaërobes of several species.

Weinberg and Séguin[1] studied carefully 126 cases of gaseous wound infection other than tetanus. Of these, 21 were gangrenous, 35 phlegmonous. Six cases of the phlegmonous type yielded only aërobic bacteria. No case of gaseous gangrene was observed to be due to aërobes alone. On the other hand, among 30 cases showing only anaërobes, 24 were of the gaseous type. Thus aërobes are apparently able to produce gaseous phlegmons, but the number of such cases is small; gaseous infections are predominantly anaërobic and the rôle of aërobes is only accessory. Ninety cases contained both aërobes and anaërobes. The flora of anaërobic wound infection is usually polymicrobic, for of the 120 cases yielding anaërobes, 50 contained a single anaërobe, 70 more than one.

From these 120 cases Weinberg and Séguin isolated the following obligate anaërobes:

	Cases.	Per Cent.
Cl. perfringens (B. welchii)	91	72
Cl. sporogenes	34	27
Cl. œdematiens (B. novyi)	33	26
Cl. fallax	26	21
Vibrion septique	12	9.5
Cl. tetani	11	8.7
Cl. histolyticus	8	6.3
Cl. aërofœtidus	5	3.9
Cl. putrificus	2	1.6
Cl. bifermentans	2	1.6
Cl. tertius	1	0.8
Bacillus II of Ghon and Sachs	1	0.8

It is evident that the bacteriology of gaseous gangrene is complicated, and furthermore, that no etiologic claims can be based upon the mere occurrence of any organism in war wounds. While, as stated by Weinberg and Séguin, there is in effect a typical form of gaseous gangrene: (1) it is not always produced by the same microbe; (2) it is frequently caused by several associated agents;

[1] Weinberg and Séguin: La Gangrène Gazeuse, Paris, 1918.

(3) it is often the complex result of the combined action of these principal agents with numerous other germs which play an indeterminate accessory rôle.

In Weinberg and Séguin's 50 mono-anaërobic cases six species were encountered, as follows:

Cl. perfringens (B. welchii)	37
Cl. œdematiens (B. novyi)	6
Vibrion septique	1
Cl. fallax	3
Cl. sporogenes	2
Cl. aërofœtidus	1
	50

One or more of these six species occurred also in every one of the 70 cases having a polyanaërobic flora; moreover, among the 12 anaërobes recovered only the 6 just mentioned produced any of the symptoms of the gaseous infection when injected alone into laboratory animals. These organisms are the "anaërobic agents of the gaseous infections."

(1) THE VIBRION SEPTIQUE

In 1877 and 1881 Pasteur,[1] while studying anthrax, produced a septicemia in rabbits and guinea-pigs by the inoculation of putrid blood from a cow. The affection could be communicated from individual to individual, and a sporulating, motile, rod-shaped anaërobe, considered by him "one of the vibrions of putrefaction," was regarded as the cause of the septicemia and named "vibrion septique."

In 1881 Koch[2] described the pathologic effects of an organism which he declared identical with the vibrion septique of Pasteur. But this organism refused to produce a septicemia in experimental animals (guinea-pigs); and since its pathogenic effects were limited largely to the site of inoculation, Koch designated it "the bacillus of malignant edema."

Neither Pasteur's nor Koch's description would suffice now to identify the organism in question with certainty. Fortunately, the original strain of Pasteur's "vibrion septique" has been maintained in France, so that its outstanding characters are well known. But the lack of any such legacy from Koch in Germany, due to his

[1] Pasteur: Bull. Acad. de méd., 1877, p. 781; 1881, 2s., 10, p. 176.

[2] Koch: Mitteil. aus dem kais. Gesundheitsamte, 1881, 1, pp. 1–49.

failure to recover cultures, has led to an all but interminable discussion as to the properties of the "true bacillus of malignant edema." Weinberg and Séguin maintain that the term "bacillus of malignant edema" is untenable and should be dropped from bacteriologic nomenclature.

The vibrion septique is a gram-positive, sporulating clostridial rod, or filament, motile in young cultures by means of many peritrichal flagella. The ends are slightly rounded and the spores, which are oval, are usually median and swell the organism into a clostridium previous to their release (Fig. 96). Spores are formed only in media not containing fermentable carbohydrates in excess. The long chains and filaments of this organism which occur on the visceral surfaces of infected guinea-pigs are of high differential value. Capsules have never been observed.

Fig. 96.—Vibrion septique. Broth culture; × 1000 (McIntosh). (From Reprint No. 39, Medical Research Committee, Special Report, 1919.)

The vibrion septique is a strict anaërobe. It develops readily in deep brain or tissue media, producing gas rather abundantly; these media are not discolored, even in the presence of metallic iron. Broth cultures become quite turbid with gas and acid production in the presence of certain fermentable carbohydrates. Deep colonies in 1 per cent. agar are transparent or semitransparent. Hemolysis occurs on blood-agar.

Gelatin is liquefied, but coagulated serum and other proteins are not digested and never blackened. Milk is acidified and the casein slowly clotted; the casein clot is not subsequently digested. Indol is not produced. Glucose, levulose, galactose, maltose, lactose, and salicin are fermented; media not containing one of these sugars support only slight growth. Saccharose, inulin, mannit, and dulcit are not fermented. Fermentation of salicin and nonfermentation of saccharose offer an opportunity for differentiation from Cl. chauvei, which it most closely resembles, for the action of Cl. chauvei upon these two substances is exactly opposite to that of the vibrion septique.

The pathogenicity of the vibrion septique is incontestable; this bacillus has been incriminated in gaseous gangrene of man by Ghon and Sachs[1] and many others. It has been recovered from gaseous infections in cattle and, indeed, may be one of several organisms responsible for blackleg, which has been considered generally a specific disease due to Cl. chauvei. Meyer[2] recovered the same organism in certain gaseous infections of hogs. The disease has been reproduced in swine by inoculation. Aside from these, many gaseous infections in domestic animals are due to the vibrion septique.

In experimental inoculation the vibrion septique cultures are strikingly pathogenic for chickens, pigeons, rabbits, guinea-pigs, rats, and mice. In such animals the organisms develop rapidly, producing gas and a reddish, serous edema. The bacteria invade the tissues and the circulation, producing a septicemia which is usually fatal within twenty-four to forty-eight hours, if at all; sublethal doses do not produce any reaction. Impression smears from the tissues, and especially from the liver, usually show elongated filaments or chains, a point in contrast with animals killed by Cl. chauvei, where the organisms are usually single. The so-called toxin obtained by some workers is at best a weak lethal agent, requiring relatively large doses to kill, and not at all comparable with the toxin of Cl. tetani.

Robertson,[3] by absorption tests, has demonstrated three subgroups of the vibrion septique among eleven cultures.

Weinberg and Séguin produced an antiserum in horses which was prophylactic, and in some degree curative. It was prophylactic for guinea-pigs in a dose of $\frac{1}{300}$ c.c. against $\frac{1}{4}$ c.c. (five fatal doses) of living culture, and for mice in a dose of $\frac{1}{4000}$ against $\frac{1}{20}$ c.c. (one fatal dose) of living culture. Its curative action was shown in a dose of $\frac{1}{4}$ c.c. of serum injected as late as five hours after the inoculation of mice with $\frac{1}{10}$ c.c. (five fatal doses) of living culture. No satisfactory data are available as to the efficacy of vibrion septique antisera in prophylaxis or therapy of pure, spontaneous vibrion septique infections.

[1] Ghon and Sachs: Centralbl. f. Bakt., Abt. 1, Orig., 1903, 34, pp. 289, 398, 481, 609.

[2] Meyer: Jour. Infect. Dis., 1915, 17, p. 458.

[3] Robertson: Jour. Path. and Bact., 1920, 23, p. 153.

(2) CLOSTRIDIUM WELCHII

Clostridium welchii was first cultivated by Achalme[1] and supposed by him to be the cause of articular rheumatism. In 1892 Welch and Nuttall[2] isolated a thick-set, non-motile anaërobe, "B. aërogenes capsulatus," from the foamy organs of a cadaver. In 1893 Fränkel recovered an identical organism, which he called "B. phlegmonis emphysematosæ," from several cases of gaseous phlegmon. In 1897 Veillon and Zuber found the same bacterium in various pathologic secretions and gave it the name "Bacillus perfringens." This last name is used generally in France and has the advantage of a binomial, but Migula in 1900 suggested "B. welchii," which is now most common in English-speaking countries.

More recent developments in the history include the subdivision of Cl. welchii by Simonds[3] into four varieties on the basis of fermentation or non-fermentation of inulin and glycerol. Three comprehensive reviews of the literature and bacteriology of Cl. welchii have appeared.[4]

Cl. welchii has a wide distribution in nature. It has been found in dust, soil, sewage, water, fish, mollusks, milk, cheese, and in various parts and secretions of man and the lower animals, especially the feces. Some writers have proposed that the presence of Cl. welchii in water should be taken as evidence of sewage pollution, but the fact that the organism occurs abundantly in soil does not countenance the adoption of such a criterion.

Cl. welchii is a plump, non-motile, gram-positive rod of variable length, occurring in chains and singly. Capsules are usually present in preparations made from the organs or body fluids. Spores are formed sparingly and only in the absence of fermentable carbohydrates; they are centrally located, rarely subterminal, and do not swell the rod in which they are formed. No flagella have been demonstrated and cultures are invariably non-motile. Motility tests should be made with young cultures (six to twenty-four hours' incubation). Cover-slip preparations using a plain slide are adequate, but negative results are more dependable if observed with a warm stage.

[1] Achalme: Comptes rend. Soc. de biol., 1891, 13, p. 651.

[2] Welch and Nuttall: Bull. Johns Hopkins Hosp., 1892, 3, p. 81.

[3] Simonds: Monograph No. 5, Rockefeller Inst. for Med. Research, 1915.

[4] Simonds: Ibid. Jablons: Jour. Lab. and Clin. Med., 1920, 5, p. 374. Esty: Jour. Bact., 1920, 5, p. 375.

Cl. welchii is a strict anaërobe. It grows readily in deep brain, meat infusion broth, agar, and gelatin media. Its growth in sugar-free media is greatly restricted. Optimum conditions of growth are provided by media containing fermentable carbohydrates, but such cultures are often short-lived owing to (*a*) the lack of spores, and (*b*) the action of acids upon the vegetative forms. Brain and meat media are not blackened normally, but the presence

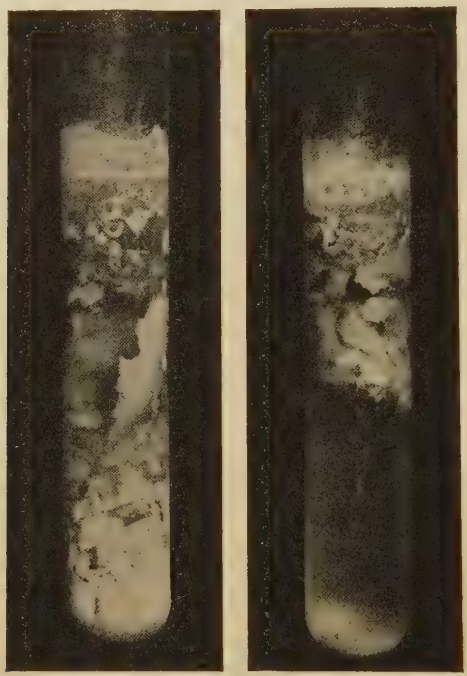


Fig. 97.—Typical reactions of Clostridium welchii in milk, forty-eight hours old (N. MacL. Harris prep.).

of metallic iron produces a distinct discoloration. Sulfuretted hydrogen is produced in small amount, but indol production is still a matter of dispute. Isolated colonies in deep, 1 per cent. agar are compact, opaque, white, or grayish-white biconvex discs.

Broth containing fermentable sugars becomes markedly turbid with abundant gas formation, and many cultures, possibly all at certain stages, become stringy and viscid. Gelatin is liquefied, but coagulated serum is not liquefied. Milk is fermented with a characteristic "stormy" evolution of gas, followed by coagulation of the casein due to acid formation, and the curd is shortly torn

to shreds by the continued evolution of gas within (Fig. 97). The curd is not digested. This "typical" reaction is considerably modified by incomplete anaërobiosis, so that one may sometimes observe slow gas production, followed by the formation of a solid curd slightly, if at all, torn by gas.

Simonds, Henry, and others have differentiated four subgroups of Cl. welchii according to their action on glycerol and inulin:

Type	Glycerol	Inulin	Number of Cultures in Simonds' Series	Number of Cultures in Henry's Series
I	+	+	4	9
II	+	−	7	4
III	−	+	5	5
IV	−	−	4	2
		Total.....	20	20

In milk, 3.8 times the volume of the milk may be evolved as gas. During the early stages of fermentation hydrogen predominates; during the later stages carbon dioxid is more abundant than hydrogen. Owing to its strong saccharolytic properties Cl. welchii is perhaps the best species to use in the preparation of sugar-free broth intended for fermentation test media.[1] Koser[2] found it to be the active agent in a commercial leaven advocated for the preparation of salt-rising bread.

Pathogenicity for Man.—The causal relationship of Cl. welchii to gaseous gangrene has been abundantly established. Simonds, before the war, collected from the literature 175 cases, with a mortality of 45 per cent., and Weinberg and Séguin found this organism in 91 (72 per cent.) out of their 126 cases. Thus, Cl. welchii is the most important of the various causes of gaseous gangrene. While "it is as a cause of that most dreaded of wound complications, emphysematous gangrene, that B. aërogenes capsulatus especially claims the interest of surgeons" (Welch), the bacillus has also been observed in closed abscesses in uterine infections, and in infections of the gastro-intestinal, genito-urinary, and biliary tracts. Several observers have isolated it from the blood during life, but septicemia in man is much less common than in experimental guinea-pigs, although blood invasion occurs frequently in man during the

[1] Randall and Hall: Jour. Infect. Dis., 1921, 29, p. 344; 1922, 31, p. 326.
[2] Koser: Jour. Infect. Dis., 1923, 32, p. 208.

agonal period or immediately following death. Study of the "foamy organs" sometimes observed at autopsy has shown that the presence of gas in the internal organs shortly after death is often attributable to an invasion by this organism. The development of gas in the liver is a striking phenomenon in many of these cases.

Herter[1] has shown that in certain forms of disease the human intestinal tract contains an excessive number of bacilli belonging to this group. The characteristic type of "saccharobutyric putrefaction" induced by Cl. welchii may perhaps give rise to products that bring about an anemic condition. Many instances of anemia in children and adults seem to be accompanied by a chronic infection of the intestinal tract by Cl. welchii, and as the general condition of the patient improves there is a distinct reduction in the numbers of this organism found in the feces.

Feeding experiments in man and animals have usually proved harmless.

Pathogenicity for Animals.—In the lower animals natural infection seems rare; local abscesses have been observed, however, in dogs and rabbits following injury. Certain strains of Cl. welchii are pathogenic under proper conditions for guinea-pigs, pigeons, and mice, less so for rabbits. Dead rabbits, however, were used as a culture-medium by Welch and Nuttall.[2] If a rabbit is killed a few minutes after intravenous injection of Cl. welchii and the body incubated at 37° C., gas is produced in a few hours throughout the body, and the phenomenon of "foamy liver" reproduced. This phenomenon is not strictly specific for Cl. welchii; it may be produced by similar inoculations with several other anaërobes, although the results are less striking.

The toxigenicity of Cl. welchii was long in dispute. Several observers demonstrated that a true antigenic, thermolabile, albeit a relatively weak, exotin is formed by Cl. welchii. Bull and Pritchett[3] emphasized three features in the production of this toxin, namely, the use of broth containing only minute quantities of glucose, the addition of raw muscle tissue, and the filtration of twenty-four-hour, 37° C., cultures. Such filtrates were hemolytic and toxic for pigeons in a dosage of 0.2 c.c. or more. The toxin is thermolabile

[1] Herter: Jour. of Biol. Chem., 1906, 2, p. 1.

[2] Welch and Nuttall: Bull. Johns Hopkins Hosp., 1892, 3, p. 81.

[3] Bull and Pritchett: Jour. Exp. Med., 1917, 26, pp. 119, 367, 603. Cf. also Williams: Brit. Jour. Surgery, 1926, 14, p. 295.

(70° C., thirty minutes), non-dialyzable, and not dependent upon acidity, since neutralization fails to destroy it. It produces hemolysis *in vivo* by intravenous injection, and inflammation, edema, and necrosis by subcutaneous or intramuscular injection. It does not produce gas in the tissue; gas must be regarded therefore as a product of metabolic activity in the growth of the organism itself in a wound. Bull and Pritchett found quantitative but not qualitative differences in the toxins formed by a total of 27 strains of Cl. welchii. The toxin is antigenic and antitoxin produced from any single strain protects against toxin produced by any other strain.

Agglutinins have been observed by several workers, but several careful investigators have failed completely to produce agglutinins even by prolonged immunization.

There are but few important references in the literature to active protective immunization against whole cultures of Cl. welchii. Robertson[1] concluded that "vaccination with killed or attenuated cultures of B. perfringens does not cause any appreciable increase in the resistance of guinea-pigs against a subsequent lethal dose of living bacilli" and that "recovery from a previous infection with the organism does not prevent a repetition of the illness upon re-inoculation with living bacilli; nor does it apparently in any way alter the symptoms or influence the course of the disease." Esty, however, produced protective immunity by the injection of sublethal doses of virulent cultures. Hall showed that the protective mechanism of immunity to Cl. welchii is not strain specific, as is the agglutination reaction, but is species specific.[2]

Bull and Pritchett actively immunized pigeons and rabbits by successive injections of carefully graded doses of toxic filtrates, and the blood-serum from the rabbits was found to neutralize the toxin *in vivo* and *in vitro* in multiple proportions and had both prophylactic and curative properties. Passive immunity in guinea-pigs lasts for about two weeks. Fortunately, antitoxic specificity for Cl. welchii relates to the species rather than to the fermentive type, thus reducing to a single strain the representation of this organism in the polyvalent sera that must be used for gaseous gangrene.

[1] Robertson: Journal-Lancet, 1916, 36, p. 388.
[2] Hall: Jour. Infect. Dis., 1922, 30, p. 445.

(3) CLOSTRIDIUM NOVYI[1]

The third important anaërobe in gaseous gangrene was probably first discovered by Novy[2] in 1894 through a study of "malignant edema" that occurred in three guinea-pigs injected with non-sterile nuclein prepared from casein, and was designated by him "Bacillus œdematis II." Weinberg and Séguin have pointed out the apparent identity of this organism with the one described by them in 1915 under the name "B. œdematiens." The specific designation Cl. novyi is used here in reference to the data accumulated by Weinberg and Séguin and others for B. œdematiens.

Clostridium novyi is noteworthy not only as the third important pathogenic anaërobe in gaseous gangrene, but also because of its strong, soluble exotoxin, which compares in potency with the toxin of B. diphtheriæ and for which an equally powerful antitoxin has been produced.

Morphology.—Cl. novyi is a rather thick (0.8–1 μ) flagellated, gram-positive rod of variable length (2.5–10 μ), occurring singly and in short chains. In cultures, navicular and curved forms are to be found; in animal transudates the shorter form predominates. Its numerous spiral flagella, which often become tangled in "bouquets," have received emphasis in nearly all the published descriptions. Yet the rods are quite non-motile under ordinary conditions of examination, their movements being markedly inhibited by air. Subterminal spores are produced readily, even in the presence of glucose.

Physiology.—Cl. novyi is a strict anaërobe, said to require an unusual depression of oxygen tension for its cultivation. For this reason it has possibly been overlooked not infrequently in analyses of mixed cultures.

Young colonies in deep glucose-agar have a yellowish, opaque, irregular center surrounded by a delicate corona of short filaments. Later the colony clears, the center becomes cloudy, and is surrounded in forty-six hours with a corona of tangled filaments. Surface colonies are extremely delicate, flattened, transparent, bluish gray, with irregular contours. Cl. novyi is slightly hemolytic.

In glucose broth the usual initial turbidity of anaërobic cultures gives way after a few hours to a striking sedimentation, followed

[1] This is probably a group of anaërobes rather than a single species (H. H. Heller).

[2] Novy: Ztschr. f. Hyg., 1894, 17, p. 209.

by autolysis and consequent clearing of the medium. Gas and acid are formed and old cultures have a characteristic fetid odor.

Abundant growth requires the presence of a fermentable sugar. Henry[1] found glucose, levulose, maltose, xylose, and starch fermented. There is not complete agreement as to the fermentation reactions; a recent report[2] states that glycerol, galactose, saccharose, lactose, mannit, dulcit, inulin, and salicin are not fermented.

Cl. novyi can be easily distinguished from the vibrion septique and Cl. chauvei, which it most closely resembles, by its nonfermentation of lactose, its formation of a strong specific soluble exotoxin, and, to a degree, by the character of its lesions in animals.

Pathogenicity for Man.—Weinberg and Séguin first showed the great importance of Cl. novyi as an active agent in human gaseous gangrene. It was present in 13 per cent. of the first 100 cases reported by them. A later report gives an incidence of 26 per cent. (33 out of 126) and, in 12 per cent. of these, death was attributed directly to this organism owing to its purity in the infections (3 cases) or association with less pathogenic anaërobes. The disease is characteristically a toxemia, although septicemia is not rare. Like Cl. welchii, Cl. novyi is often a terminal invader. In pure infections there is less tissue destruction than with Cl. welchii or the vibrion septique; the postmortem findings consist mainly in a massive localized edema, with neither the extensive gas production of the former nor the sanguineous necrosis of the latter. These facts are in accord with its nature as a former of strong exotoxin.

Pathogenicity for Animals.—Natural infections due to Cl. novyi have been recorded for guinea-pigs, cattle, horses, and hogs. Guinea-pigs, rabbits, rats, mice, cats, sheep, horses, and pigeons are susceptible to small doses of culture. Subcutaneous, intramuscular, and intravenous inoculations reproduce the disease experimentally.

The action of the toxin freed from bacteria is very similar to that of whole cultures. Sublethal, subcutaneous doses of toxin or culture produce a peculiar non-hemorrhagic local edema which reaches its maximum in two to three days. It may be followed by an appearance of small, superficial hemorrhages, after which it is slowly absorbed, leaving a slightly sclerotic scar. Such lesions appear not to form open phlegmons, as in the case of Cl. welchii

[1] Henry: Jour. of Path. and Bact., 1917, 21, p. 344.

[2] Bullock: Medical Research Committee, Special Report Series, No. 39, 1919.

cultures, and may also be contrasted with those of the vibrion septique and Cl. chauvei, which, if they appear at all, are always fatal. Washed cultures are harmless.

Agglutinating antisera have been produced in rabbits, but are active for homologous strains alone.

Antitoxin has been produced in rabbits, sheep, and horses by successively increased doses of toxic filtrates. Tested in guinea-pigs, 0.0001 c.c. was found to neutralize 2 fatal doses (0.02 c.c.) of toxin; 100 fatal doses (2 c.c.) were neutralized by 0.004 c.c. of serum. The antitoxin possesses prophylactic and to some extent therapeutic properties under experimental conditions.

(4) BACILLUS HISTOLYTICUS

Among the new species of bacteria discovered by Weinberg and Séguin[1] in war wounds, none is of more interest than B. histolyticus, so named because of its remarkable liquefying action upon living tissues. It occurred in 8 instances of wound infection out of the 30 last cases studied by them. It has also been recovered from soil and human feces in California.[2]

Morphology.—B. histolyticus is a gram-positive motile rod that forms subterminal clostridial spores. In smears from lesions it appears generally in the form of single or paired short rods with rounded ends. The flagella, often more than 20 in number, are peritrichal.

Physiology.—Described originally as obligately anaërobic, B. histolyticus has more recently been found capable of a delicate transparent aërobic growth upon the surface of meat infusion agar slants.[3] It is therefore properly designated as a microaërophile or facultative anaërobe. Deep agar colonies vary, according to the consistency of the medium, from compact lobulate globules in 2 per cent. agar to fluffy semitransparent or even cottony balls in lower concentrations. Under anaërobic conditions surface colonies on blood-agar slants are hemolytic dew drops; aërobically there is little or no hemolysis.

Coagulated proteins, such as egg, muscle and brain tissue, are slowly digested, and the species should be regarded as distinctly

[1] Weinberg and Séguin: La Gangrène Gazeuse, Paris, 1917.

[2] Peterson and Hall: Proc. Soc. Exp. Biol. and Med., 1923, 20, p. 502; Hall: Proc. Soc. Exp. Biol. and Med., 1923, 21, p. 198.

[3] Hall: Proc. Soc. Exp. Biol. and Med., 1923, 20, p. 501.

putrefactive. But the proteolytic activity is mild *in vitro* as compared with its tremendous digestive action *in vivo*. Gelatin is liquefied. The action on milk is slow, but after several days a soft clot is usually formed and then slowly digested. Blanc and Pozerski[1] have shown that a proteolytic ferment is formed. White crystals, thought to be tyrosin, are produced in old protein cultures. No carbohydrates are known to be fermented and all cultures remain neutral or become alkaline. There is also only a slight amount of gas produced in cultures, or none at all.

Pathogenicity.—No instance of pure infection by B. histolyticus occurring naturally has been recorded. As generally happens in war wounds, Weinberg's and Séguin's cases were polymicrobic. The associated bacteria were both aërobic and anaërobic pathogens and saprophytes. Similar infections were observed in equines, and such mixtures show enhanced virulence in experimental animals.

Most pure cultures of B. histolyticus are pathogenic under experimental conditions for rabbits, guinea-pigs, mice, and rats, but there is considerable difference between strains. Subcutaneous inoculation of 1–2 c.c. of a twenty-four-hour broth culture generally produces a local tumefaction followed in twenty-four to forty-eight hours by complete sloughing of the overlying skin; healing then slowly occurs, as a rule. Intramuscular inoculation causes swelling, followed by progressive myolysis. If the gluteus muscle of a guinea-pig is selected for the inoculation, it may be entirely denuded from the bone within twenty-four to forty-eight hours. The tissues literally drip or are licked away. In some cases the limb may be disarticulated. Curiously there is often little or no intoxication of the animal, but death usually follows through peritonitis due to perforation of the peritoneum. There is occasionally invasion of the blood-stream, but generally septicemia does not occur. There is never any gas formation in such pure infections.

Germ-free culture filtrates have a lytic action which can be demonstrated if sufficiently large quantities (5 c.c.) are injected. The most characteristic effect is the formation of a sterile hematoma filled with uncoagulated blood in which the red corpuscles are still intact.[2]

[1] Blanc and Pozerski: Compt. rend. Soc. Biol., 1920, 83, p. 1369.

[2] Peterson and Hall: Proc. Soc. Exp. Biol. and Med., 1923, 20, p. 504.

Weinberg and Séguin have produced agglutinating and antitoxic serums experimentally.

(5) CLOSTRIDIUM SPOROGENES

Clostridium sporogenes, which in pure culture must be regarded as a relatively harmless organism, is included in the present discussion for three reasons: first, its historic interest in connection with the confusion long existing between the vibrion septique and the "true bacillus of malignant edema"; second, the important symbiotic rôle played by this and other proteolytic anaërobes in association with non-proteolytic organisms, and third, the great frequency of these organisms in nature.

It is useless to speculate upon the nature of the organisms concerned in Koch's "malignant edema," since specific bacteria were not isolated by him, and our present knowledge indicates that several species were probably concerned in the pathology with which he dealt. Adamson[1] and Heller[2] give a clear exposition of the historic confusion connected with Cl. sporogenes.

There is no question that Cl. sporogenes is one of the most widely distributed obligate anaërobes in nature. Adamson found it present in the feces of 7 out of 15 men, in the feces of the horse, and in garden soil. Many writers have recovered this or similar organisms from soil and water. It is apparently the most frequent anaërobic contaminant of laboratory cultures of anaërobes. Hall found Cl. sporogenes in cultures masquerading under such names as Cl. welchii (5 strains), Cl. chauvei (1 strain), Cl. botulinum (4 strains), Cl. feseri (1 strain), Cl. tetani (2 strains), and in many of these there was no evidence of the presence of the organism indicated by the label. These findings indicate one reason for the misconceptions and confusion in much of the older literature on anaërobes.

Morphology.—Cl. sporogenes is a highly motile, gram-positive, somewhat slender rod occurring individually, in pairs, in short chains, and sometimes in filaments.

Spores are formed with especial facility in sugar-free media, but occur also in media containing fermentable carbohydrates. They are subterminal, oval in shape, and clostridial in form. Chains of

[1] Adamson: Jour. Path. and Bact., 1919, 22, p. 345.
[2] Heller: Jour. Infect. Dis., 1920, 27, p. 385.

rods occur in which some of the individuals bear spores and others do not, or they may all bear spores. In such chains the spores of any two adjacent rods may be proximal, alternate, or distal.

The resistance of the spores is very high, a fact that partly accounts for the great frequency with which Cl. sporogenes is isolated by methods involving selective heating, as well as the survival of this organism in many stock cultures of anaërobes bearing other names.

Physiology.—Cl. sporogenes requires strict anaërobiosis for growth, but its nutritional requirements are somewhat less fastidious than those of the non-putrefactive anaërobes. All of the methods of cultivation described in these pages are readily applicable to this species.

Isolated colonies in soft (1 per cent.) deep agar are translucent and woolly in appearance with a compact center; in harder agar the whole colony tends to be compact. Surface colonies on blood-agar are transparent and usually rhizoid, or ameboid with a slightly raised center; they appear moist and at first may resemble minute dewdrops. They are hemolytic.

Deep brain or meat media give rise to gas formation (hydrogen and carbon dioxid) and a characteristic proteolytic digestion associated with a darkening or even blackening of the medium near the surface. An excess of fermentable sugar inhibits or delays this phenomenon; the presence of metallic iron or certain iron salts accelerates it. Gelatin and coagulated proteins are liquefied. In all media containing proteins the odor gives evidence of putrefaction by Cl. sporogenes. Among the products of digestion, sulfuretted hydrogen, ammonia, amino-acids, and volatile acids are prominent. Indol production is in doubt.

Reports on the fermentation of various carbohydrates are conflicting. The gross action of Cl. sporogenes on milk is characteristic of several other putrefactive anaërobes as well. Growth at first is slow. Within forty-eight to seventy-two hours a soft clot is formed with little or no gas production. From that time on, a progressive liquefaction occurs with abundant gas and acid formation. After several weeks a milk culture appears to consist of a clear, straw-yellow fluid with a sediment in the bottom, the clotted casein having been completely digested. The cultural features of Cl. sporogenes closely resemble those of Cl. botulinum.

Pathogenicity.—There is no authentic record of a natural infection attributable to Cl. sporogenes alone. Weinberg and Séguin seem inclined to accept Metchnikoff's claim that Cl. sporogenes is a factor in certain intestinal disorders, but the frequent occurrence of this organism in the intestinal tract of man and other animals seems against any such conclusion.

In experiments on animals rather large doses are required to produce lesions; less than 5 c.c. of a young glucose broth culture injected subcutaneously in guinea-pigs, which are most susceptible, usually causes only a local manifestation. In a few hours the hair immediately overlying loosens, the skin becomes gangrenous and raised slightly over an area of subcutaneous tissue digestion in which a small amount of gas appears. Such animals usually show no systemic involvement and the lesion heals in a few days, leaving a necrotic scar which heals slowly. Intramuscular injections are only slightly more severe.

The most obviously important of the pathogenic manifestations of Cl. sporogenes (and probably of other putrefactive anaërobes) is that of a mutual acceleration in metabolism which occurs during growth with the more definitely pathogenic anaërobes, especially Cl. welchii, the vibrion septique, and Cl. novyi. Stimulating as is the presence of various aërobes to the growth of obligate anaërobes, due partly, as Pasteur suggested, to the absorption of oxygen, but also to other unknown factors, the presence of putrefactive anaërobes greatly enhances the pathogenicity of the non-putrefactive pathogens. Drawing an analogy between the action of high explosives and the violent exhibitions of gaseous gangrene, certain bacterial forms might be said to act as "detonators." The putrefactive anaërobes supply in great abundance split protein products which the more highly saccharolytic organisms are unable to elaborate so rapidly. But while an admixture of a putrefactive culture reduces the minimum fatal dose of a non-putrefactive anaërobe experimentally, the admixture of sporogenes filtrate with toxic filtrates of Cl. welchii or Cl. novyi has no such action; in fact, there may be a destructive action.

Blackleg

Blackleg, also known as quarter evil and symptomatic anthrax, is an important, wide-spread, acute disease affecting cattle. It

occurs at practically every altitude of the world where cattle are kept and is prevalent throughout the United States with the possible exception of the Southern Atlantic and Eastern Gulf States. The name blackleg, like gaseous gangrene, has been applied to affections due to different microbes. In some cases the vibrion septique or rarely Cl. novyi are found, but the principal cause is **Cl. chauvei.** Just as Cl. welchii has never been incriminated in infections of

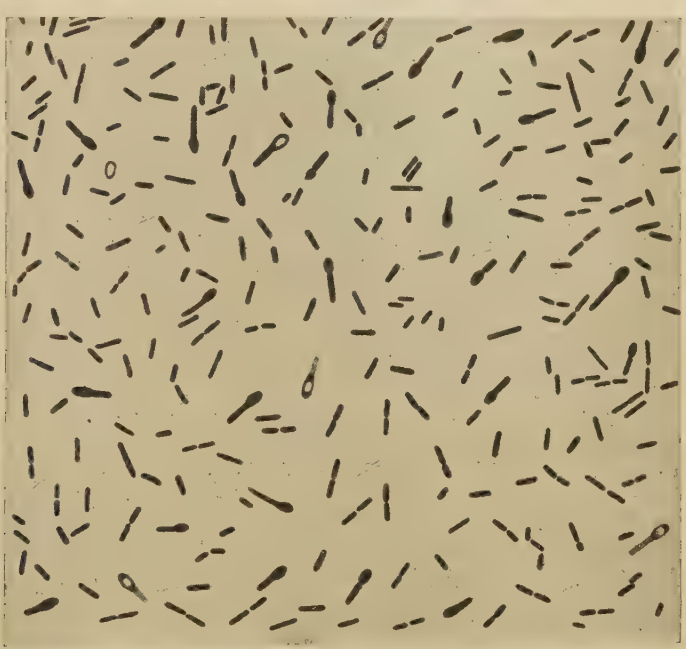


Fig. 98.—Clostridium chauvei from twenty-four-hour culture in dextrose agar, gram stain (Ford, "A Text-book of Bacteriology").

quadrupeds, so Cl. chauvei has never been shown to be responsible for any human infection.

Bollinger, in 1875, and Feser, in 1876, observed the characteristic organisms in the crepitant swellings, and transmitted the disease by injecting the serous fluid from these swellings into healthy animals. The most important of the early contributions were those of Arloing, Cornevin, and Thomas,[1] who firmly established the etiology in the cultivation of the organism now regarded as the principal cause, which they named Cl. chauvei, and with which they produced the characteristic disease by inoculation.

[1] Arloing, Cornevin, and Thomas: "Le Charbon symptomatique du bœuf," Paris, 1887.

Cl. chauvei is a gram-positive, motile, sporulating rod of variable size. Individuals usually occur singly; in contrast with the vibrion septique there is little tendency to form chains or filaments. The spores are usually subterminal, oval, and clostridial, swelling the rod in which they occur. Sporulation is often preceded by a marked swelling of the vegetative rod (Fig. 98).[1]

Cl. chauvei is strictly anaërobic. Deep colonies in agar are minute, compact, and downy. On the surface of blood-agar slants, under alkaline pyrogallol, well separated colonies are flat, round or leaf-like, and hemolytic. By far the best medium is a ground meat or brain medium, in which rather abundant gas gives evidence of rapid multiplication. These media are never discolored or digested by pure cultures, but they may be slightly softened. Other coagulated proteins are not liquefied, but gelatin is liquefied.

Glucose, levulose, galactose, maltose, saccharose, and lactose are fermented, while inulin, salicin, mannit, dulcit, and glycerol are not fermented. The exactly opposite reactions of the vibrion septique on saccharose and salicin serve to differentiate this closely related form.

Pathogenicity.—Natural infections due to Cl. chauvei occur principally in cattle. There are still several obscure features, relating especially to the epidemiology. The disease occurs at special seasons of the year, is connected with certain localities, and is said to show a distinct predilection for the best young stock. The portal of entry is uncertain; whether it is minute wounds of the skin or through the gastro-intestinal mucosa is quite unknown. The work of Meyer[2] and Heller also reopens the question whether Cl. chauvei causes spontaneous disease in domestic animals other than the cow.

Experimentally, Cl. chauvei is pathogenic for cattle, sheep, goats, guinea-pigs, and mice; horses, asses, hogs, rabbits, rats, and pigeons are somewhat refractory.

The symptoms of naturally, as well as of artificially, infected animals consist in crepitant localized swellings, which occur on the thighs, neck, or shoulders of naturally infected animals. Such animals are stupid, feverish, and anorectic. Treatment is rarely successful and sick animals usually die in from one to two days.

[1] Heller: Jour. Infect. Dis., 1920, 27, p. 385.

[2] Meyer: Amer. Vet. Rev., 1915, 47, p. 684; Jour. Infect. Dis., 1915, 17, p. 458.

The mechanism of the disease is that of a progressive bacteremia. Toxin production has been reported by some European workers, but American bacteriologists have not confirmed their statements.[1]

Arloing, Cornevin and Thomas, of Lyons, France, invented a means of prophylactic inoculation which has been widely used in active immunization. The Lyons vaccine, as distributed by the Bureau of Animal Industry of the United States Department of Agriculture,[2] is prepared as follows: The muscle tissue from a fresh blackleg tumor is pulverized in a mortar, extracted with a little water, and the fluid squeezed through a piece of cloth. This is then dried at 35° C. The dry brown scale which results is suspended in water and injected in appropriate quantities as determined by tests and specified on each package distributed. The dried material retains a high degree of virulence for several years and can at any time be mixed with water (2 parts), heated for six hours at 95° to 99° C., and inoculated. Some commercial firms distribute this vaccine in the form of strings to be sewn into the flesh; others dispense the powdered vaccine in the form of pellets which are inoculated by means of a "pill gun." In 1901–02 (July 1st to July 1st) 565,628 cattle were vaccinated in the United States; during the previous season 14,817 deaths had occurred; in a similar period after vaccination the number of deaths was only 2902. Franklin and Haslam[3] have shown that some of the commercial preparations are seriously contaminated and lack protective power.

Roux was able to immunize guinea-pigs by injecting the edema fluid, expressed from the flesh of guinea-pigs dead of blackleg and rendered bacteria free by filtration through a Pasteur-Chamberland filter. Roux suggested that such a product might have value in the control of blackleg, and this was shown to be possible experimentally by Schöbl. In tests involving over 5000 guinea-pigs single prophylactic doses of 10 c.c. were found to protect over 83 per cent. against five lethal doses of a virulent culture. The field results are said to be highly promising.

The use of "culture filtrate" in immunization also traces back to Roux (1888). Kelser[4] gives the details of manufacture, which

[1] *E. g.*, Scott: Jour. Infect. Dis., 1926, 38, p. 262.

[2] Norgaard: U. S. Dept. of Agric., Bur. of Animal Indus., Circ. No. 31, 1907.

[3] Franklin and Haslam: Jour. Infect. Dis., 1916, 19, p. 408; 1920, 26, p. 424.

[4] Kelser: Jour. Agric. Research, 1918, 14, p. 253.

involve securing a bacteria-free filtrate from Cl. chauvei cultures in a ground meat peptone bouillon.

Goss[1] states that during twelve consecutive months in 1918–19 39,880 doses of blackleg filtrate (including "culture filtrate" and "tissue filtrate") were sent out by the Kansas Agricultural Experiment Station without a single loss from blackleg among inoculated stock being reported. The impression is prevalent, however, that, of the two preparations, the tissue filtrate is more efficacious.

Specific agglutinins can be produced by certain strains of Cl. chauvei which are active at least toward the homologous cultures.

Botulism

Botulism is a form of food poisoning characterized by great muscular weakness due to paralysis of the motor nerve centers. Failure of ocular accommodation, drooping of the eyelids (ptosis), difficulty in swallowing (dysphagia), loss of voice (aphonia), excessive salivation, or in some cases dryness of the mouth and throat owing to salivary inhibition, obstinate constipation, and retention of urine are among the symptoms. In contrast with the intestinal *food infections*, gastro-intestinal symptoms are usually not prominent. There is rarely fever; unimpaired consciousness without ability of expression is the rule. The case mortality is high, death being ascribed to asphyxia of bulbar origin. Recovery, if it occurs, is complete but tedious; visional disturbances and muscular weakness persist for a long time.

Botulism was long regarded as a rarity in America, but numerous cases have been recognized since 1912, and a retrospective review of the literature indicates that many instances of so-called "ptomain poisoning" were, in reality, botulism.

Botulism was apparently first definitely observed in Germany in 1735, but the disease did not attract much attention until the latter part of the 18th century, when a number of outbreaks following the eating of pig stomach and other large sausages earned for it the name (Lat., botulus, a sausage) now recognized as not altogether appropriate.

The German poet and medical writer, Justinus Kerner,[2] in 1820 and 1822 enumerated 174 cases with 71 deaths which had occurred

[1] Goss: Kans. Exp. Sta. Circ., 1919, 75, p. 4.

[2] Jordan: Food Poisoning, Univ. of Chicago Science Series, 1917.

in various epidemics in Württemberg since 1793. Three reasons were assigned for the apparently universal prevalence at that time: first, the great consumption of large sausages; second, the use of inferior materials in their manufacture, and third, primitive methods which failed to provide sufficient smoking. Mayer's report in 1913[1] showed that botulism has continued to be prevalent in Germany, as follows:

1822–1886: 238 cases, 94 fatal (about 39 per cent.).
1886–1913: About 800 cases, about 200 fatal (25 per cent.).

Most of the German cases were associated with the eating of sausages, especially the larger sizes; meat sausages were less frequently implicated than blood and liver sausages. But other hog products, pickled and smoked hams and salted pork, have been implicated and there are records of cases in Russia and Denmark, due to the eating of smoked and salted fish, which indicate botulism.[2]

The true etiology of botulism was established by van Ermengem following an epidemic at Ellezelles, Belgium, in 1894, where 23 persons became ill after eating of a pickled ham, and 3 died. From the residue of the ham and from the spleen and intestinal contents of one of the victims van Ermengem isolated the causative organism, which he named Bacillus botulinus.

It is now known that botulism is endemic in the United States, that the toxin may be formed in purely vegetable media, that limberneck in fowls is botulism, and that *thrombosis* is a characteristic feature of the pathology. Since it has been shown also that forage poisoning in horses may be caused by Cl. botulinum, the name *botulism* has lost its original significance as related to the disease in man and is even less appropriate for animals.

The wide-spread occurrence of outbreaks in man and animals implies a wide distribution of this organism in nature. Previous to 1919 Cl. botulinum had been recovered from natural sources other than foods and cadavers only once, when Kempner and Pollack[3] in 1897 recovered it from the feces of a hog. Before this van Ermengem had failed repeatedly in making cultures from garden soil, street dust, pond mud, stable manure, cow, horse, and duck feces, and intestinal contents of fish. Dickson too was unsuccessful subsequently in his attempts to confirm Kempner and Pollack

[1] Jordan: Food Poisoning, Univ. of Chicago Science Series, 1917.
[2] Dickson: Rockefeller Inst. for Med. Res., Monograph No. 8, 1918.
[3] Kempner and Pollack: Deut. med. Wchnschr., 1897, 23, p. 505.

through the examination of a large number of hog feces. But Burke[1] isolated Cl. botulinum from fruits, hay, and vegetables, from the bodies of insects and spiders, and from the droppings of a hog that had recovered from botulism. Meyer and Dubovsky[2] have found Cl. botulinum in soil samples from all parts of the United States and from many European countries. It seems to be more abundant along the Pacific Coast than in the Mississippi Valley and Great Lakes region.

CLOSTRIDIUM BOTULINUM

Morphology.—Cl. botulinum is a large, pleomorphic, gram-positive, motile, sporulating rod, occurring singly, in pairs, and in chains (Fig. 99). Its size is usually 4 to 6 μ by 0.9 to 1.2 μ, but the dimensions are quite variable. There are 4 to 8 peritrichal flagella. The spores are subterminal and oval in shape. Some strains produce them abundantly, others sparsely.

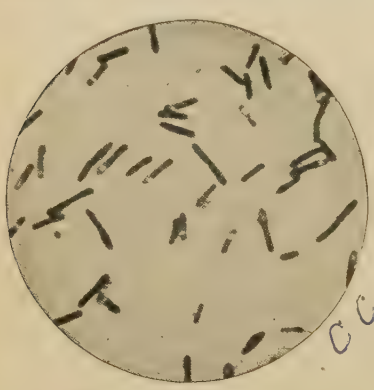


Fig. 99.—Clostridium botulinum, with spores. Pure culture. Van Ermengem prep. (Kolle and Wassermann).

Physiology.—Cl. botulinum is an anaërobe, a fact which is of importance in interpreting its relation to food poisoning. Only preserved foods—not fresh foods—are concerned in the production of botulism, and only such methods of preservation as exclude oxygen. Large sausages, canned goods and silage fulfil these conditions.

Van Ermengem considered growth to occur only at room temperature, and on that account spoke of Cl. botulinum as a pathogenic saprophyte. It is now established that both growth and toxin production occur at 37° C. Indeed, growth is more rapid at the latter temperature.

Growth is readily obtained in the usual media for anaërobes. Glucose broth is clouded, acidified, and gives rise to gas. Deep colonies in agar are translucent and globular and diffuse, or flat and

[1] Burke: Jour. of Bact., 1919, 4, p. 541.

[2] Meyer and Dubovsky: Jour. Infect. Dis., 1922, 31, p. 557; Ibid., p. 600

heart shaped or disk shaped, according to the consistency of the medium. Burke[1] distinguishes a type of colony peculiar to Cl. botulinum, with an inclusion of minute gas bubbles.

Brain, meat, and other coagulated protein media are blackened and digested. Gelatin is liquefied. The reaction upon milk exactly resembles that of Cl. sporogenes; there is slow coagulation of the casein, followed by rapid digestion accompanied by gas production. The organism is predominantly proteolytic. Sulfuretted hydrogen is produced abundantly; indol production is distinct in certain cultures. Nitrates are rapidly reduced by certain strains. Monosaccharids are fermented, but not higher carbohydrates directly. Growth ceases in 6 per cent. salt solution, but not in 25 to 50 per cent. syrup. The critical P_H is around P_H 4.9.

Resistance of the Spores.—It is obvious that Cl. botulinum may with considerable facility find entrance to food during or previous to preservation, and that the preservative processes should be such as to inhibit development and destroy the most resistant spores.

Since the majority of botulism cases in this country have been due to the use of underheated ("underprocessed") foods the chief practical problem is the determination of the maximum heat resistance of botulinum spores and the application of sufficient heat to insure their destruction. It is essential to obtain proper heat penetration and to allow for altitude.[2] Esty and Meyer[3] have shown that the heat resistance of 109 tested strains varied greatly. The maximum heat resistance of Cl. botulinum spores in a phosphate solution of P_H 7.0 was as follows:

4 minutes at 120° C.
10 minutes at 115° C.
33 minutes at 110° C.
100 minutes at 105° C.
330 minutes at 100° C.

Young, moist spores, probably those of the first generation, appear to be the most heat resistant. The hydrogen-ion concentration affects thermal resistance. Heat increases the permeability of the spores to carbol fuchsin. The long-continued dormancy of

[1] Burke: Jour. of Bact., 1919, 4, p. 555.
[2] The temperature of boiling water at Denver is only about 95° C.
[3] Esty and Meyer: Jour Infect. Dis., 1922, 31, p. 650.

certain spores has an important bearing on conclusions drawn from experiments on heat resistance.[1]

Toxin.—The most important metabolic product of Cl. botulinum is the powerful botulism toxin. It appears to be produced whenever abundant growth occurs, irrespective of the presence of fermentable carbohydrates. Formerly supposed to be formed only in media containing animal protein, Dickson[2] first showed that these may be replaced by proteins wholly of vegetable origin.

Botulism toxin differs from any other known bacterial toxin in that it is not destroyed by the gastro-intestinal secretions. Extremely potent when tested either orally or parenterally, a culture filtrate subcutaneously injected frequently kills guinea-pigs in a dose of 0.0001 c.c.; when the filtrate is fed to guinea-pigs 0.001 c.c. often proves fatal. Brieger and Kempner obtained a toxin of which 0.000,001 c.c. would kill a 250-gram guinea-pig. Cl. botulinum may be likened to certain of the higher plants, such as poisonous mushrooms, the deadly night-shade, and the fungus of ergot (*Claviceps purpurea*), which are dangerous by virtue of the poisonous compounds that are generated in their cells or in the substances in which they proliferate.

The toxin is thermolabile and actinolabile. Van Ermengem found the toxicity of filtrates much diminished by heating at 50° C. for three hours, and destroyed by 80° C. for thirty minutes, and by boiling for at least ten minutes. It is insoluble in alcohol, ether, and chloroform. It is destroyed by strong alkalis, but is resistant to moderately strong acids. Indeed Bronfenbrenner and Schlesinger[3] found its potency enhanced by acidification, but Geiger and Gouwens[4] were unable to confirm this.

Antigenically there are two distinct types of botulism toxin produced by separate races of Cl. botulinum, which are otherwise so far as known indistinguishable. One of these races of Cl. botulinum (Type A) was found most commonly by Meyer and Dubovsky[5] in the soil of the Rocky Mountain and Pacific Coast states, while Type B was found more often in the Mississippi Valley,

[1] G. S. Burke: Jour. Infect. Dis., 1923, 33, p. 274.

[2] Dickson: Jour. Amer. Med. Assoc., 1915, 65, p. 492.

[3] Bronfenbrenner and Schlesinger: Jour. Amer. Med. Assoc., 1922, 78, p. 1519.

[4] Geiger and Gouwens: Pub. Health Reports, 1923, 38, p. 2249.

[5] Meyer and Dubovsky: Jour. Infect. Dis., 1922, 31, p. 559.

Great Lakes region, and Atlantic Coast states. A toxin-producing anaërobe isolated principally from fly larvæ possesses group characters similar to those of the other two types, but the toxin that it produces is neutralized only by the antitoxin derived from homologous strains. The fly larva type has a lower thermal death point than Types A and B and in this respect resembles the original van Ermengem strain. It has been called Type C by Miss Bengtson.[1]

Pathogenicity.—Natural cases of human botulism always follow the eating of preserved foods in which the organism has grown and produced its specific toxin. Many vegetable foods have been implicated, fish and various pork products, in addition to sausages. The first record in the European literature in which a food not of animal origin (string beans) was thought to be responsible occurred in Darmstadt, Germany, in 1904,[2] and it was suggested in this case that some pork must have been cooked with the beans, as otherwise the toxin of botulism could not have been formed. In France, botulism is said to be very rare, and in Great Britain, the first known outbreak occurred in 1922.[3] In the United States a number of human cases suggestive of botulism were on record previous to 1914, but Wilbur and Ophuls in that year reported an outbreak due to home-canned beans which was but the first of many since recorded for such products, including beans, corn, spinach, asparagus, pears, apricots, and beets.

Prior to 1919 there was no well authenticated record of botulism attributable to commercially canned goods. Spoilage in tin cans due to anaërobes is usually detected with ease in the "swells" which are eliminated before such products are sold. Weinzirl and Cheyney found living organisms, mostly sporulating aërobes, in merchantable canned goods, but although only a few obligate anaërobes and no pathogenic organisms were detected in their selected material, Burke[4] suggested that the possibility of botulism being conveyed by commercially canned foods was by no means ruled out.[5]

The first authentic instances of botulism due to commercial products in the United States were outbreaks due to contaminated

[1] I. A. Bengtson: Pub. Health Repts., 1923, 38, p. 340.
[2] Cited by Dickson: Jour. Amer. Med. Assoc., 1915, 65, p. 492.
[3] Gerald Leighton: "Botulism," London, 1923, pp. 237.
[4] Burke: Jour. Amer. Med. Assoc., 1919, 73, p. 1078.
[5] Editorial, Jour. Amer. Med. Assoc., 1919, 73, pp. 1844, 1887.

California ripe olives in 1919–20. Up to January 1, 1924, there have been 129 outbreaks affecting human beings in the United States, all but three of these having occurred since 1909; 435 persons were affected in these outbreaks and there have been 290 deaths, a case-mortality of 67 per cent. The majority of the outbreaks in which the faulty food was identified, were due to preserved vegetables. Precautions taken in the food-preserving industries seem to have been generally efficacious. Since February, 1922, one outbreak of botulism has been traced to a commercially canned food, while in this period there have been 26 instances attributed to home-canned foods.

While Cl. botulinum is a putrefactive micro-organism, it is not always easy by the senses to detect spoilage in foods in which it has grown. This is especially true of fruits and vegetables containing an excess of fermentable carbohydrates whose fermentation might tend to precede or indeed inhibit putrefactive processes. In many outbreaks unusual flavors have been noticed and commented upon; in others there has been no evidence whatever of spoilage. The housewife's principal safeguard lies in eternal vigilance coupled with an invariable practice of preheating (boiling ten minutes) all canned goods before serving. Testing foods by taste is a dangerous practice. In some cases housewives have been made extremely ill, and, indeed, have died from tasting such foods in order to test their quality.

Pathogenicity for Animals.—Associated with human cases have been numerous outbreaks among fowls fed spoiled home-canned foods. Hart reported a single outbreak among 800 fowls of which 643 died after feeding upon garbage to which spoiled home-canned beans had been consigned. Dickson records a total of 22 such outbreaks involving either chickens, turkeys, or hogs. Recent reports also indicate that horses, cattle, and goats can contract botulism.

Rabbits, guinea-pigs, mice, apes, cats, and dogs are susceptible to experimental injection or feeding of toxin. The symptoms are similar to those of naturally infected animals. At autopsy an intense congestion of the internal organs, especially of the lungs, is observed, and microscopic examination reveals degenerative changes in the nerve-cells of the bulb and cord and in the salivary glands. Very small doses of the poison provoke local paralysis

and lead to a cachectic condition which in some animals ends fatally after weeks or months.

Wilbur and Ophuls[1] and Dickson have emphasized the predominance of circulatory disturbances as evidenced in extensive thrombosis and hemorrhagic lesions. Dickson and Shevky[2] have proved that there is neither any effect upon the sensory fibers of the peripheral nerves, nor upon either the smooth or the striated muscle cells, but that there is a block in certain parasympathetic motor fibers as well as upon the nerve endings of the voluntary system.

Edmunds and Long[3] consider that the essential action of the botulinum toxin is a more or less complete paralysis of the motor nerve-end plates in the striated muscles and in the diaphragm.

Immunity.—Kempner in 1897 first produced botulinum antitoxin in goats, 1 c.c. of serum protecting guinea-pigs against 100,000 minimum lethal doses.

Kempner failed to immunize rabbits actively, but Forssman and Lundstrom[4] succeeded with both guinea-pigs and rabbits by starting with heat-attenuated toxin.

The net result of these attempts has been to confirm the conclusion of Leuchs[5] that there are two distinct antigenic types of botulinum toxin. It is remarkable that while there is no known constant morphologic or cultural distinction in the organisms producing these separate types of toxin, and no apparent difference in their action upon the animal body, they appear to be as distinct antigenically as diphtheria toxin and tetanus toxin.

More than four times as much antitoxin is required to neutralize a given test dose of toxin *in vivo* as *in vitro*. The antitoxin is to some degree effective when swallowed.

All investigators of botulinum antitoxin have been able to protect experimental animals against fatal doses of toxin providing sufficient antitoxin was administered *previous to the onset of symptoms, but not afterward*. The situation is similar to that in tetanus, except that there is relatively little opportunity to predict the occurrence of botulism in a given individual. Dickson has sug-

[1] Wilbur and Ophuls: Arch. of Int. Med., 1914, 14, p. 589.
[2] Dickson and Shevky: Jour. Exp. Med., 1923, 37, p. 711; 1923, 38, p. 327.
[3] Edmunds and Long: Jour. Amer. Med. Assoc., 1923, 81, p. 572.
[4] Forssman and Lundstrom: Ann. de l'Inst. Past., 1902, 16, p. 294.
[5] Leuchs: Ztschr. f. Hyg., 1910, 65, p. 55.

gested that in some cases the death of chickens from limberneck might serve as a warning and indication for the use of antitoxin in persons who may have tasted the suspicious food before it was consigned to the chickens.

There are no well controlled data showing any curative value of antitoxin in natural cases of botulism, although antitoxin has been used a few times, and should be administered always in the hope of turning the balance in favor of recovery in borderline cases.

CHAPTER XXII

THE TUBERCLE BACILLUS

Although it is still true that few diseases are so wide-spread or occasion so much distress and economic loss as tuberculosis, recent progress in combating this disease has been highly encouraging. In the United States tuberculosis mortality has shown an almost continuous decline since 1911, the death-rate for the registration area falling from 159.2 per 100,000 population in 1911 to 86.6 in 1925. It can hardly be doubted that much of the decline in this disease occurring in the past few decades should be credited to improvement in living conditions and to the extension of sanatorium treatment.

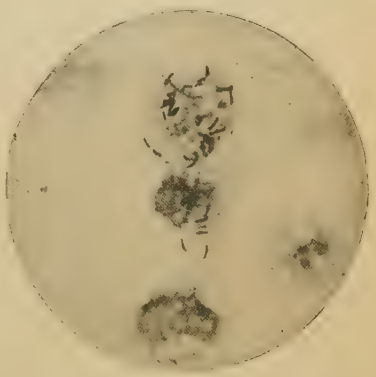


Fig. 100.—Bacillus tuberculosis, human, in pus from lung. Zettnow prep. (Kolle and Wassermann).

The fact that tuberculosis is a specific inoculable disease was shown by Villemin[1] as early as 1865, but Robert Koch in 1882 first established the etiology of tuberculosis on a solid basis.[2] Koch succeeded: (1) in demonstrating by special staining methods that the tubercle bacillus was present in a great variety of affected organs and tissues; (2) in securing pure cultures of the bacillus in the face of great technical difficulties; (3) in performing successful inoculation experiments with the isolated cultures.

Morphology.—In film preparations, made either from sputum or from cultures, the human tubercle bacillus (Fig. 100) ordinarily appears as a slender rod, often slightly curved, about 2 to 4 μ long and 0.3 to 0.5 μ broad. The individual rods may occur singly, but often lie in small heaps. A capsular substance is produced by tubercle bacilli in artificial cultures; it is more abundant in human

[1] Villemin: Gaz. hebdom., 1865, 2 S., 5.

[2] Koch: Berl. klin. Wchnschr., Apr. 10, 1882, 19, p. 221.

than in bovine strains, and the amount becomes greater with the length of artificial cultivation on serum. In cultures also a remarkable filamentous growth has been observed, and in sputum long branching, hypha-like filaments, sometimes with swollen ends, have been found. By some observers these clubbed and branching forms are regarded as abnormal or involution forms, possessing no significance in the normal life of the tubercle bacillus. Others, and perhaps the majority, consider that the branching filaments seen in cultures represent normal morphologic characters of the tubercle micro-organism. In the animal body likewise a stellate actinomyces-like (p. 530) growth is sometimes produced by the tubercle bacillus. Some bacteriologists, on the basis of these findings, class the tubercle bacillus either with the trichomycetes (Ch. XXVIII) or with the true molds, while others place the tubercle bacillus and certain closely allied micro-organisms in a special group holding an intermediate position between the ordinary bacilli and the trichomycetes. Whatever be the final outcome of taxonomic discussion, the tubercle bacillus with its near allies must be regarded as standing rather apart from most other pathogenic bacilli, and possibly may eventually turn out to be "the parasitic growth-form of a higher mold." It must be added that certain observers claim that a filterable stage of the tubercle bacillus occurs.

The minute structure of the tubercle bacillus has been the object of much discussion. Vacuoles often occur so abundantly as to give to the rod the appearance of a chain of cocci, and these unstaining spaces have been sometimes mistaken for spores, especially by the earlier students of the organism. More recently several observers have described small, deeply staining bodies within the cell, which in some of their morphologic and tinctorial characters resemble the spores formed by other bacteria. These spore-like bodies, however, display little or none of the heightened resistance to the action of heat and chemicals which is so characteristic a feature of other bacterial spores. The true nature of these structures is, therefore, still problematic. The tubercle bacillus is non-motile, and no flagella have been seen.

Staining.—The tubercle bacillus stains very imperfectly or not at all with the ordinary aqueous anilin dyes, a fact that doubtless delayed the discovery of its presence in the tissues. If, however, the action of the dye is intensified by the aid of a mordant or by

heat, the bacilli take on a deep coloration. Once stained, they retain the color tenaciously, even when treated with alcohol or strong mineral acids. This behavior is so characteristic that the tubercle bacilli, together with certain other related organisms, are usually designated as "acid-fast bacilli." Some investigators, however, have described forms or phases of the tubercle bacillus which are not acid-fast.[1]

The examination of sputum for the tubercle bacillus is sometimes carried out by the use of antiformin. This liquid, which is composed of a mixture of 7.5 per cent. sodium hydroxid with sodium hypochlorite in such an amount that 100 grams of antiformin can liberate 5.3 grams of chlorin, was first utilized by Uhlenhuth[2] in bacteriologic studies. The intense oxidizing power of antiformin enables it to dissolve many kinds of organic matter and to destroy most forms of micro-organisms. The organisms of the acid-fast group, however, resist the dissolving action, probably because they are enveloped in a waxy capsule. Hence mixing antiformin with sputum leads to a complete dissolution of the mucus at the same time that it leaves unaltered the staining qualities of the tubercle bacillus. The method of procedure is as follows: The sputum is best placed in a conical glass and, if very tenacious, diluted with distilled water known to be free from acid-fast organisms. Add one-fourth its volume of antiformin and stir thoroughly, if necessary increasing the amount of distilled water and antiformin until complete solution is effected. When complete digestion is brought about, add an equal volume of 95 per cent. alcohol, stir, and allow the mixture to stand for eighteen to twenty-four hours. Cover-slip films may be made from the sediment in the usual way. By use of antiformin the tubercle bacillus has been frequently demonstrated in sputum that has given negative results when examined by other methods.

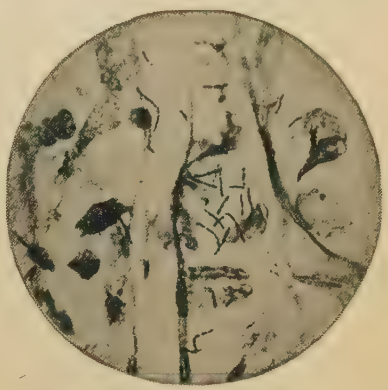


Fig. 101.—Tubercle bacilli in phthisical sputum (Beck).

[1] See v. Behring, "Tuberculosis," 6, No. 9.

[2] Uhlenhuth: Ber. klin. Wchnschr., 1908, 45, p. 1346.

Staining may be accomplished by the use of Ziehl's carbol-fuchsin solution (p. 52), the cover-slip being flooded with stain, which is boiled for one-half minute. Follow this treatment by decolorization with acid alcohol (2 per cent. HCl in 80 per cent. alcohol), and contrast staining with methylene-blue (Fig. 102). The peculiar resistance of the tubercle bacillus to decolorization seems to depend upon a relative impermeability of the cell-body to acids. The constituent of the cell especially responsible for this behavior is an alcohol, mykol ($C_{29}H_{56}O$?), which is perhaps in part present in the

Fig. 102.—Bacillus tuberculosis in sputum, Ziehl-Gabbett; × 650 (Cornet and Meyer).

bacterial body as an ester of some higher fatty acid.[1] It has been claimed that other kinds of bacteria can be made to acquire "acid-fast" qualities by being grown upon media containing fatty ingredients. When tubercle bacilli are mechanically disintegrated the specific staining reaction is lost.

Cultivation.—It is not easy to cultivate the tubercle bacillus directly from tuberculous lesions. Growth fails to take place on

[1] Tamura: Ztschr. f. physiol. Chem., 1913, 87, p. 85.

ordinary nutrient agar or gelatin, and at first is slow and scanty even on the most favorable media. After cultivation has once succeeded, transplantation to another tube of the same medium will result in a more abundant growth, and in subcultures growth will take place upon media on which the bacillus fresh from the animal tissues fails to proliferate.

Koch first succeeded in cultivating the tubercle bacillus by the use of inspissated blood-serum, and this medium still remains one of the most satisfactory for isolation. Once cultures are obtained they can be grown on a variety of substrata. Glycerol-agar (Nocard and Roux[1]), prepared by adding 2 to 5 per cent. of glycerol to ordinary nutrient agar, has been widely used. Upon this medium subcultures can be made and a more luxuriant growth secured than by the continued use of serum; glycerol-agar, however, is not suitable for effecting a primary isolation. Glycerol broth is a favorable medium for well-established cultures and allows the development of a heavy growth. Care must be taken that the layer of broth is shallow and fosters a surface growth, since the tubercle bacillus requires an abundant supply of oxygen. Glycerolated potato is used by many French bacteriologists.

Dorset's simple egg medium[2] is a satisfactory medium for maintaining continuous growth. Fresh, thoroughly cleansed eggs are broken into a sterile flask and the yolks and whites thoroughly mixed without frothing. The medium is placed in sterile tubes, about 10 c.c. in each, and hardened in the inspissator in a slanted position by heating on two successive days from four to five hours at 70° C.

The modified egg medium devised by Petroff is today commonly used for isolation. Petroff's medium is prepared with meat extract (500 grams veal or beef to 500 c.c. of 15 per cent. glycerol in water, extracted for twenty-four hours, pressed out and sterilized) and whole egg with the addition of gentian violet.

Meat extract (as above)	100 c.c.
Whole egg	200 "
1 per cent. alcohol solution gentian violet	1 "

Tube and heat for three-fourths hour at 80° to 85° C. on three successive days.

[1] Nocard and Roux: Ann. de l'Inst. Past., 1888, 1, p. 19.
[2] Dorset: Amer. Med., 102, 93, p. 555.

Various synthetically prepared media have been used by Kühne[1] and others. Proskauer and Beck[2] found growth to occur in a solution of the following simple constitution:

Ammonium carbonate	0.35 per cent.
Mono-potassium phosphate	0.15 "
Magnesium sulfate	0.25 "
Glycerol	1.5 "
Water	97.8 "

Recent observers have found that after the original isolation the tubercle bacillus grows well in a variety of simple synthetic media. Glycerol, however, seems to be essential for good growth (Long). Ammonium oxalate, succinate and tartrate and other ammonium salts promote growth. There is nothing distinctive in the mineral requirements of the tubercle bacillus.

Fig. 103.—Bacillus tuberculosis, source human. Mature colony on glycerol-agar. Actual size (Swithinbank and Newman).

Biologic and Chemical Characteristics.—Upon the surface of blood-serum and glycerol-agar colonies of the tubercle bacillus appear in about ten days as minute, barely visible grains. They are dull and dry in appearance and irregular in outline. In cultures fresh from the tissues the colonies usually remain small and separate without confluence, but in subcultures, especially upon glycerol-agar, the growth is more luxuriant and the surface of the medium becomes covered with a wrinkled film (Fig. 103). The color of the growth is a lusterless white, often becoming faintly tinged with brown or yellow in old cultures. On egg medium the colonies ultimately become confluent and the growth is rather profuse. In glycerol broth growth may occur in separate patches on the surface, or may form a continuous, heavily wrinkled pellicle. Sometimes masses of bacilli sink to the bottom of the fluid as a lumpy sediment. A peculiar almond-like odor is often noticeable.

Whatever the medium employed, the temperature range within which growth occurs is a narrow one. The best development occurs between 37° and 38° C.; growth usually ceases above 42°

[1] Kühne: Ztschr. f. Biol., 1894, 30, p. 221.

[2] Proskauer and Beck: Ztschr. f. Hyg., 1894, 18, p. 128.

and below 28°, although in glycerol-potato broth Sander[1] found growth taking place at a temperature as low as 22° or 23° C. Unlike most bacteria, the tubercle bacillus grows better on media of a slightly acid reaction; this is another feature in which it shows affinity with the molds or hyphomycetes.

The chemistry of the tubercle bacillus has received particular attention at the hands of investigators. An extensive and valuable critical review of the chemistry of tuberculosis has been given by Wells, DeWitt, and Long.[2] The waxy substance in the cell, to which the tubercle bacillus owes its characteristic staining qualities, is believed to be, in large part, either an alcohol or a combination of certain fatty acids (chiefly palmitic acid) with the higher alcohols. The tubercle bacillus differs from the majority of bacteria in having a relatively high lipin content, perhaps 40 per cent. of the dry weight of the bacillus consisting of fat-like substances. The bulk of the lipin is the wax to which reference has been made.

About half the dry weight of the tubercle bacillus is of protein nature, and a considerable portion of the protein is combined in the form of nucleo-proteins. The nucleic acid of the tubercle bacillus is of the animal and not the plant type. The ash varies with the composition of the culture medium.

Powers of Resistance.—Although multiplication of the tubercle bacillus can take place, as a rule, only within a narrow range of conditions, the vitality of the bacillus under adverse circumstances is considerably greater than that of most pathogenic bacteria. In putrefying sputum it may occasionally remain viable for weeks or even months. Musehold[3] has found it in the soil of sewage fields and in sewers connected with sanitaria for consumptives. Living tubercle bacilli have also been found in river-water contaminated by sewage from a health resort.[4]

Considerable resistance to desiccation is shown. The bacilli in masses of dried sputum kept in a cool dark place may retain their virulence for as long as six to eight months, but not for much longer. Sputum that is completely dried, so that particles are capable of

[1] Sander: Arch. f. Hyg., 1893, 16, p. 238.

[2] H. G. Wells, L. M. DeWitt, E. R. Long: "The Chemistry of Tuberculosis," 1923, pp. 447.

[3] Musehold: Arb. a. d. k. Gesund., 1900, 17, p. 56.

[4] Brown, Petroff, and Heise: Amer. Jour. Pub. Health, 1916, 6, p. 1148.

floating as dust in the air, may be infective for eight to ten days, rarely longer.

Toward dry heat the bacilli are highly resistant, being able, in dried sputum, to withstand a temperature of 100° C. for an hour. When they are heated while suspended in a fluid, such as water, broth, or milk, Theobald Smith has shown that death occurs in fifteen to twenty minutes at 60° C. If, however, milk is heated at this temperature in an open vessel, the pellicle that forms in contact with the air may protect the bacilli against a temperature of 60° for as long as an hour. Pasteurization, to be effective, therefore, must be carried out in a closed vessel at 60° C. for twenty minutes. Boiling for five minutes completely destroys the vitality of the bacilli. As is the case with most bacteria, extreme cold is not germicidal.

Carbolic acid (5 per cent. solution) added to sputum requires a long time (twenty-four hours) to kill the bacilli because of its slow penetration. Lysol, which has a solvent action on mucus as well as strong germicidal power, is to be preferred. Gastric juice, because of its acidity, impedes development, but does not kill all bacilli introduced into the stomach; an experiment has been recorded in which the gastric juice of a dog did not destroy the vitality of tubercle bacilli in eight hours.

Exposure to direct sunlight readily effects the destruction of tubercle bacilli, especially in the presence of abundant oxygen supply. The conditions of exposure determine the time necessary to produce death, but, broadly speaking, bacilli from cultures are killed in a few minutes to two hours, while bacilli in sputum require twenty to thirty hours, or even longer.

Tuberculous Infection in Man.—Practically every organ and tissue of the human body may be invaded by the tubercle bacillus. As is well known, the lungs constitute the seat of the most common lesions, but the intestines and mesenteric glands, the serous membranes, the larynx, the skin, the lymph-glands of the head and neck, the portal glands and liver, the bones and joints, and the urogenital system are frequently attacked. In ordinary cases of tuberculosis there is no conclusive proof of the frequent and continued presence of tubercle bacilli in the blood. Statements of positive findings seem to be due to the presence of acid-fast bacilli in the distilled water used for making the microscopic examination.[1] Tubercle

[1] Brem: Jour. Amer. Med. Assoc., 1909, 53, p. 909.

bacilli are sometimes present in the gall-bladder and make their way into the intestine with the bile; they may occur in feces in considerable numbers. Lesions caused by the tubercle bacillus, in whatever part of the body they occur, usually possess a definite although not absolutely characteristic appearance and histologic structure. Small nodules or tubercles, plainly visible to the naked eye, are so uniformly observed in all advanced infections with the tubercle bacillus that their presence has given the name to the disease. The young tubercle according to the opinion of most investigators, originates from the fixed cells surrounding the invading bacilli. By the proliferation of the fixed cells, elongated or "epithelioid" cells are developed in more or less definite concentric layers and come to form the substance of the tubercle. So-called giant-cells soon appear in the developing tubercle in the majority of cases. The giant-cells are huge multinuclear masses of protoplasm, of oval or irregular shape, and have been held to be especially distinctive of true tubercle formation, although it is doubtful if this criterion can be maintained. As a rule, a giant-cell is produced by the fusion of a number of epithelioid cells, although some observers maintain that it is of single cell origin. While the formation of epithelioid and giant-cells is going on, leukocytes (at first polymorphonuclear leukocytes and later lymphocytes) which have wandered out of the blood vessels cluster around the periphery of the tubercle. Degeneration of the tubercle eventually sets in, the central portion becomes necrotic, and this is followed by caseation and then by softening of the caseous mass. Finally, in many cases a deposit of calcium salts takes place in the tubercle, converting it into a hard, dry, friable body which may become encapsulated and completely walled off from the surrounding tissue.

The early stages of tubercle formation, which are characterized by cell proliferation and leukocytic infiltration, are probably to be referred to a chemical or mechanical stimulus caused by the presence of the bacilli; the later changes, leading to cell necrosis and caseation, are reasonably attributed to the action of the bacterial products. The tuberculin reaction, that is, the interaction between antigen and highly sensitized tissues, certainly plays a great part in causing necrosis. The hardening, drying and shrinking of a tubercle probably betoken a healing process. Coalescence or conglomeration of tubercles commonly takes place, the confluent masses sometimes

reaching a diameter of 4 or 5 centimeters. In severe cases there occurs a general diffusion of small tubercles of the size of millet-seeds (acute miliary tuberculosis). This form of the disease seems to depend upon the simultaneous or nearly simultaneous discharge of large numbers of bacilli into the blood-stream, an event that may be brought about by erosion of the vessel wall by a tuberculous process approaching from without, or in other ways.

The work of Prudden and Hodenpyl,[1] Straus and Gamaléia,[2] and others, has established the important fact that the injection into the circulation of dead tubercle bacilli in considerable numbers can lead to typical tubercle formation. The substance present in the dead bacteria that produces this reaction is not destroyed by high temperatures, as shown by the positive results obtained after exposure of the bacilli to 115° C. for ten minutes. In these experiments giant-cell formation takes place in apparently the same way as under the stimulus of living bacilli, but caseation develops, when at all, only to a slight degree, and is never extensive.

Long[3] has given the following excellent description of the chain of chemical factors involved: "In the progression of tuberculosis we have the tubercle bacillus as the first link, a wax-armored microorganism, maintaining itself in necrotic tissue, picking and choosing its nutriment from the heterogeneous mass set before it, utilizing the glycerol of hydrolyzed fats, and probably building its wax therefrom, taking ammonia from certain of the amino-acids produced in the digestion of dead protein, utilizing others directly to speed up the process of synthesis of its own protein, autolyzing to a slight extent, sufficiently to sensitize the surrounding host to its diffusible protein products, being carried by the lymph, by phagocytes or otherwise, to new soil, there to be met by a non-specific foreign body response, which in the end operates to produce anemia and death of the isolated cells. Then we have the failure of that dead tissue to autolyze, perhaps because of the presence of ferment-inhibiting substances within the bacillus, the phenomenon of caseation. Finally, there is more or less absorption of foreign protein from that focus, that of the bacillus itself and that of the disintegrating tissue, both toxic to the body protoplasm, both capable of causing fever and

[1] Prudden and Hodenpyl: N. Y. Med. Jour., 1891, 53, pp. 637, 697.

[2] Straus and Gamaléia: Arch. méd. expér., 1891, 3, p. 705.

[3] E. R. Long: The Biochemistry of Tuberculosis, Bull. Johns Hopkins Hosp., 1922, 33, p. 246.

stimulating the metabolism of the host, so that in severe cases the typical picture of consumption ensues."

Tuberculous Infection in the Lower Animals.—(*a*) *Mammalian Tuberculosis.*—Among the larger domestic animals, cattle and swine are most commonly found affected. The horse is very rarely attacked, and sheep also appear to be relatively exempt. Slaughter-house statistics in Europe show that about 15 to 30 per cent. of cattle and about 2 to 3 per cent. of swine are tuberculous. In the United States, as far as the figures are available, the proportion of tuberculous animals slaughtered in abattoirs is not nearly so great as in Europe. According to the reports of the United States Bureau of Animal Industry, 28,000,000 cattle were subjected to postmortem examination during the years 1900–1905, and 0.134 per cent. were found to be affected. The large proportion of range cattle that have led an open-air life is probably responsible for this favorable showing. In countries where the delicate tuberculin test has been systematically applied a larger number of animals is found to react than is indicated by the results of slaughter-house inspection.

Animals in menageries and zoölogical gardens frequently die of tuberculosis. The smaller laboratory animals, as a rule, are susceptible to artificial inoculation, although not contracting the disease under natural conditions. In experimental work the guinea-pig is chiefly used.

The anatomic and clinical features of bovine tuberculosis are in the main similar to those of human tuberculosis, but show also some differences, such as the earlier calcification of the tubercles and the extraordinarily slow progress of the disease. When the pleuræ are affected, nodules appear in greater or less abundance on the visceral or parietal surfaces, a condition that constitutes the so-called pearl-disease (Ger., *Perlsucht*) of cattle.

The important question whether the tubercle bacilli found in bovine, human, and other varieties of mammalian tuberculosis are identical—whether, in other words, a single type of micro-organism is responsible for all the various forms of mammalian tuberculosis—is in a certain sense not ripe for solution. The features that should characterize a "bacterial race or species" have not yet been established, and until some consensus on this point has been reached, discussion of specific identity is futile. As often

presented, the issue is simply one of potential transformation. The subject of types of tubercle bacilli has received particular attention in connection with the relation between bovine and human tuberculosis. There are certain slight but constant differences between the bacilli isolated from human sputum and tissue lesions and those of bovine origin. The bacilli obtained from cattle are shorter, straighter, and thicker than those obtained from man; they can be cultivated less readily, as a rule, on artificial media, and they are uniformly much more virulent for rabbits (Theobald Smith). In broth containing at least 3 per cent. of glycerol, the reaction produced by human cultures is at first alkaline and then quite strongly acid, while with bovine cultures a slight initial alkalinity is produced and the cultures then remain at or near the neutral point (Theobald Smith).[1] When a large series of cultures is studied, irregular and atypical reactions are met with, so that the reaction curve in glycerol broth, although broadly agreeing with the degree of virulence for rabbits, is not a final and absolute criterion for separating the bovine and human varieties.[2]

There is no doubt, from the experiments of Smith, Koch, and others, that cattle can be less readily infected with human tubercle bacilli than with cultures from bovine sources. In itself this does not prove a specific distinction any more than the limited number of successful inoculation experiments that have been reported by other investigators prove a specific identity. The possibility must be kept in mind that bovine tubercle bacilli, after a sojourn in the body of man, may become altered not only in minor morphologic and cultural characters, but in virulence for cattle. On the whole the evidence at hand indicates that the tubercle organisms found in the bodies of different species of mammals are so closely related that it is permissible to speak of them together as "the bacilli of mammalian tuberculosis," but it is also true that the question whether the different morphologic and physiologic varieties are biologically permanent "races" or are mutually convertible is an open one. It is still uncertain how far tubercle bacilli of bovine type may be modified by residence in the human body. There are some observations which show a pronounced stability of type.

[1] Smith, Theobald: Trans. Assoc. Amer. Physicians, 1903, 18, p. 108; Jour. Med. Res., 1905, 13, pp. 253, 405.

[2] Grund: Jour. Med. Res., 1911, 25, p. 335.

Hess[1] has recorded two cases in which bovine tubercle bacilli lived in human cutaneous tissues for six to seven years without acquiring characteristics of the human type.

(*b*) *Avian Tuberculosis.*—Tuberculosis is one of the commonest diseases of the barnyard. Chickens particularly, and also pheasants, turkeys, and pigeons, suffer from this disease; ducks and geese are exempt.

The bacillus of chicken tuberculosis is similar morphologically to the bacillus of bovine tuberculosis, and resembles the latter organism also in its staining reactions. Culturally certain differences are usually manifest, such as a more luxuriant development, together with a softer, less crumbly consistency, and an ability to grow at higher temperatures (43° to 45° C.). The avian bacillus also grows more rapidly. The pathogenicity of the organism is quite different from that of bovine tuberculosis, the guinea-pig showing much greater and the rabbit less, resistance than to the mammalian type. Hens and pigeons, which succumb readily upon inoculation with the bacillus of avian origin, are infected with difficulty, if at all, with the bovine bacillus. On the other hand, the avian tubercle bacillus has been shown to cause tuberculosis in calves. There is evidence that the avian bacillus is of great importance in the causation of swine tuberculosis, a large proportion of the natural infections in these animals being due to bacilli of the avian type.

Among the more important attempts to transform the mammalian into the avian tubercle bacillus may be mentioned that of Nocard,[2] who introduced the mammalian bacillus in a collodion sac into the peritoneum of a fowl and observed a general approximation toward the avian type. Koch and Rabinowitsch, in the course of an extensive study of the bacilli found in the tuberculous lesions of birds, were unable to demonstrate any constant difference from the bacilli of mammalian tuberculosis.[3]

(*c*) *Tuberculosis of Cold-blooded Animals.*—A disease affecting carp has been found to be apparently caused by a micro-organism somewhat resembling the tubercle bacillus in its reaction to stains and in other qualities. This microbe is pathogenic for frogs.

[1] Hess: Jour. Amer. Med. Assoc., 1909, 53, p. 916.

[2] Nocard: Ann. de l'Inst. Past., 1898, 12, p. 561.

[3] Koch and Rabinowitsch: Virchow's Archiv, 1907, Supplement to Vol. 190, p. 246.

The assertion has been made by some investigators that when the mammalian tubercle bacillus is introduced into the body of frogs it gradually acquires the characteristics of the piscine bacillus. Most investigators have failed to confirm such a transformation. Bacteria similar to the tubercle bacillus have also been found in snakes, lizards, and other cold-blooded animals; they seem to resemble more nearly the acid-fast "dung" bacilli than the bacilli of the mammalian type. A serologically distinguishable group of acid-fast bacilli characteristic of cold-blooded animals does not exist.[1]

Channels of Infection.—(*a*) *Respiratory Tract.*—It is known that tubercle bacilli are discharged commonly from the bodies of consumptives (1) in the sputum and (2) in minute droplets of moisture or mucus which are projected into the air by the act of sneezing, coughing, or talking. There is no reason to believe that the human tubercle bacillus flourishes saprophytically outside of the human body, and this fact, together with the existence of a large number of consumptive persons in every community, points to a more or less frequent and direct communication of the disease through inhalation. Many facts, epidemiologic and other, support this view.

The sputum of consumptives often contains an enormous number of germs. It has been estimated, from carefully obtained data,[2] that a patient suffering from phthisis may expectorate from 500,000,000 to 3,000,000,000 tubercle bacilli in twenty-four hours. Although not all of these germs possess vitality at the start, and under ordinary conditions in the open many of the discharged bacilli quickly perish from the influence of desiccation and sunlight, some may retain their vitality for several months. Bacilli are not detached from moist surfaces even by a strong current of air, so that only those bacilli that survive long enough to appear in the dust resulting from the dried sputum have much significance in infection. From the foregoing facts it is evident that the danger from inhalation of sputum bacilli is much greater in rooms, offices, public conveyances, and workshops frequented by consumptives than in the open country or even in dust-laden city streets. Careless expectoration upon the floor or into a handkerchief is the main cause of peril from this source. It can be readily seen how a

[1] Furth: Jour. Immunol., 1926, 12, p. 273.
[2] Nuttall: Bull. Johns Hopkins Hosp., 1891, 2, p. 67

handkerchief upon which sputum has been allowed to dry in the pocket, away from the influences of light and air, may be the means of spreading infection when again flourished in the air in a room, shop, street-car, or anywhere in the neighborhood of other persons. The existence of "infected houses"—a term used by several observers to characterize dwellings in which a succession of cases have appeared in the families of different tenants—witnesses to the evils of careless expectoration in closed apartments.

It is a common observation that in the act of speaking, coughing, or sneezing minute drops of fluid are violently projected from the mouth. The researches of Flügge and his pupils indicate that a certain proportion of the droplets expelled by consumptives contain living and virulent tubercle bacilli. These infectious droplets may float in the air for some time (thirty minutes?), and render the immediate neighborhood of the coughing patient more or less of a menace. The droplets are found rather rarely at a greater distance than 3 or 4 feet from the patient, and the danger of infection from this source is, therefore, circumscribed both in space and time. The relative importance of droplets and dust in producing infection is a matter of dispute. Practically, both possibilities must be taken into consideration.

Postmortem studies by Opie[1] and others have shown that in the United States nearly all persons acquire in childhood tuberculous pulmonary infections which occur as foci scattered throughout the lung without special predilection for the apices. These pulmonary lesions are almost constantly accompanied by tuberculous lesions in the adjacent lymph-nodes often more extensive than the primary lesions. In one series of 86 cases reported by Opie only 7 showed no calcified lesions on Roentgen-ray examination, and it was thought that uncalcified cicatrices probably could have been found in these. Lesions—usually multiple—were found in the remaining 79 cases, and in 10 of them the involvement of the lungs and lymph-glands was very extensive.

(*b*) *Infection Through the Alimentary Tract.*—This pathway of infection has come into greater prominence through the investigations of recent years. Tubercle bacilli may conceivably find their way into the mouth in many ways. There is evidently some danger when children soil their fingers by creeping or playing on the floor,

[1] E. L. Opie and H. Anderson: Am. Rev. Tuberc., 1920, 4, p. 629.

or when the common drinking-cup or the imperfectly cleansed spoon and fork are used. In fact, transference to the hands and food may take place from a variety of contaminated objects. Food may sometimes be contaminated through the agency of insects. It has been shown that flies can ingest tuberculous sputum and subsequently excrete virulent bacilli during a number of days.[1] The possibility of repeated self-infection of a consumptive from swallowing his own sputum may be mentioned as a peril to be avoided.

The milk and butter of tuberculous cattle are the articles of diet chiefly suspected of causing tuberculous infection by way of the alimentary tract. It has long been known, from the results of feeding and inoculation experiments, that the milk of tuberculous cattle is sometimes infectious. Tuberculosis of the mammary gland occurs in the cow much more frequently than in the human mother; from 2 to 8 per cent. of tuberculous cows manifest udder infection. It has been shown that not only milk drawn from a diseased udder is liable to contain tubercle bacilli, but also milk from an udder showing no macroscopic or microscopic lesion. Several observers have found that the milk of cattle which react to the tuberculin test, but present no other sign of the disease, *even on autopsy*, is infective. In many, perhaps all, of these cases the soiling of the udder with feces is responsible for the presence of the tubercle bacillus in the milk (Ostertag). Schroeder and Cotton[2] have, in fact, concluded that feces are the most dangerous factor in the dissemination of tubercle bacilli by cattle affected with tuberculosis. The available evidence seems to indicate that tubercle bacilli are not eliminated in the milk from tuberculous cows unless the udder or surrounding parts are themselves diseased; but, in practice, there is the possibility of contamination of the milk after it has been shed, especially contamination with feces containing tubercle bacilli.

Butter made with cream from tuberculous animals may likewise contain living bacilli for as long as one hundred and fifty-three days.[3] Gasperini[4] added tubercle bacilli to butter and found the butter virulent after one hundred and twenty days.

[1] Lord: Boston Med. and Surg. Jour., 1904, 151, p. 651.

[2] Schroeder and Cotton: Bull. 99, Bureau of Animal Industry, U. S. Dept. Agri., 1907.

[3] Mohler, Washburn, and Rogers: Bull. 41, Hyg. Lab., Washington, 1908.

[4] Gasperini: Baumg. Jahresb., 1890, 6, p. 271.

Meat from tuberculous cattle, although muscle tissue itself does not ordinarily contain tuberculous lesions, may become contaminated during removal from the carcass by being smeared with material from an infected gland coming in contact with the knife or cloth used by the butcher. Feeding experiments upon animals show that raw meat is a much less infective material than raw milk. So far as human infection is concerned, the general use of thoroughly cooked meat minimizes the danger from this source. Although various food-substances derived from tuberculous animals prove infectious in animal experiments, it does not necessarily follow that they are a frequent or an ordinary source of infection in man. Considerable difference of opinion exists concerning the relation between bovine and human tuberculosis, and the importance of this question renders desirable its discussion in a separate section (p. 444).

Much interest in recent years has centered about the manner, frequency, and results of gastro-intestinal tract infection. Many experiments have demonstrated that bacilli can pass through or between intact epithelium cells and thence into the mesenteric nodes and thoracic duct. According to most observers, passage can take place without leaving any trace in the intestinal wall; but a few hold that some tissue change, however slight, is always produced. It appears to be true that in experimental work enormous numbers of bacilli are necessary to produce infection. The final localization or site of predilection of the bacilli entering the lymphatic system from the intestines is a matter of great importance. Von Behring maintains that the vast majority of all cases of lung tuberculosis are of intestinal origin, and there is no doubt that pulmonary tuberculosis can originate from swallowing tubercle bacilli. The work of Opie,[1] however, indicates that infection with tuberculosis occurs much less frequently by way of the gastro-intestinal tract than by way of the lungs, to judge from the relative infrequency of calcified mesenteric nodules. This form of tuberculous infection appears more common among the British than in this country (*e. g.*, St. Louis, Opie), a fact that perhaps points to a more common consumption in the British Isles of milk containing living bovine tubercle bacilli. It is interesting to note that pulmonary tuberculosis lesions are infrequent or absent among those in whom mesenteric lesions occur.

[1] E. L. Opie: Am. Rev. Tuberc., 1920, 4, p. 641.

(*c*) *Inoculation.*—Infection may take place by direct cutaneous or subcutaneous inoculation of tubercle bacilli, either by accidental infection at postmortem examinations, giving rise to the so-called "pathologists' warts," or in other ways. Considerable resistance is shown by man to infection by this route, and the results of skin infection are rarely serious. Ravenel,[1] however, has recorded cases in which accidental inoculation of man with bacilli of bovine origin has resulted in serious lesions.

The Relation Between Bovine and Human Tuberculosis.—Widely divergent views are held respecting the danger to man of infection from bovine sources. On the one side there are those who, like von Behring,[2] maintain that the common forms of tuberculosis usually originate in infancy through intestinal infection with milk, and that the bacilli remain latent in the tissues for long periods; on the other, there are eminent authorities (Koch) who have declared that the susceptibility of man to bovine tuberculosis is slight, and that infection with the milk, butter, or flesh of tuberculous animals is a very rare occurrence. The question is complicated and its experimental solution presents many technical difficulties.

The difference between tubercle bacilli from bovine and human sources has already been mentioned. It was first clearly established by the work of Theobald Smith that the bovine tubercle bacillus was a distinct variety or race of the mammalian tubercle bacillus, and as such possessed certain definite and rather constant characters. Some modifications may occur in passing from host to host, but Smith believes that "the modification of bovine bacilli in the same human body beyond recognition as such by the bacteriologist, if it ever occurs, necessarily presupposes a prolonged sojourn, probably of years at the shortest."[3] Granting a fair degree of constancy, it follows that tubercle bacilli recovered from the human body may be identified with more or less certainty as of bovine or human origin. A number of observers have encountered bacilli of the bovine type in the human subject, and it is therefore plain that, so far as this line of evidence goes, infection with bovine bacilli can and does take place. At the same time, it must be noted that

[1] Ravenel: Proc. Path. Soc., Philadelphia, 1900, 3, p. 259; 1902, 5, p. 87.
[2] von Behring: Deut. med. Wchnschr., 1903, 29. p. 689.
[3] Smith, Theobald: Med. News, 1902, 80, p. 343.

the fully studied and established cases of this sort are few in number, and that there is still inadequate information respecting the relative frequency with which bacilli of the bovine type are found in human tuberculosis.

Inoculation experiments have shown that cattle may be infected with human tubercle bacilli and sputum, contrary to the view at one time enunciated by Koch.[1] Evidence that the converse also may occur is not lacking. Accidental wound inoculation of veterinarians with bovine bacilli has resulted in typical localized subcutaneous tuberculosis, which in some cases has become generalized and led to a fatal termination (Ravenel, *et al.*). These facts, however, important though they are, do not prove that the food-products from tuberculous cattle constitute a common and ordinary means of infection.

For various reasons it is peculiarly difficult to secure data as to the frequency of intestinal infection. Ingested bacilli can sometimes pass through the intestinal wall without leaving visible trace of their passage, and may finally lodge and proliferate at some point far removed from their portal of entry. Tubercle bacilli have been found in the thoracic duct shortly after they have been fed to an animal in large numbers.[2] Pulmonary tuberculosis can therefore undoubtedly sometimes arise secondarily after invasion of the body by way of the intestine. Harbitz[3] has demonstrated the frequent occurrence of tubercle bacilli in the lymph-nodes of children, and his observations have added emphasis to the frequency of primary infection through the digestive tract. In such cases, however, it is impossible to determine from anatomic data whether the bacilli are contained in the food taken and are of bovine origin, or whether they are bacilli of human origin that have entered the mouth and lymph-nodes. Hence the peculiar importance of the differential characters of bovine and human bacilli already referred to. A number of instances are on record where all the available evidence, bacteriologic and epidemiologic, indicated that the milk of tuberculous cows was responsible for the causation of tuberculosis in children. Infection from this source seems especially liable to occur when the intestine is flooded with enormous numbers of

[1] Koch: Address at British Congress on Tuberculosis, 1901.
[2] Ravenel: "Medicine," Detroit, 1902, 8, pp. 529, 617.
[3] Harbitz: Jour. Infect. Dis., 1905, 2, p. 143.

tubercle bacilli, as may happen in the use of milk from cows suffering from bad cases of udder tuberculosis.

Now that it is recognized that the milk from tuberculous cattle can serve as a vehicle for infection, it has become possible to estimate the precise share such infection has in producing the total amount of tuberculosis in any community. The only way in which this question can be settled is by the collection of data showing the number of cases of tuberculous infection in which the bovine tubercle bacillus is present. Especially important studies in this direction were made by Park and Krumwiede.[1] As the result of the examination of 478 cases of all forms of tuberculosis in New York City, these investigators conclude that a noteworthy percentage of cases of tuberculosis in children under 5 years of age are due to infection with the bovine bacillus.

Summing up all the evidence, it appears that bovine tuberculosis is a slight, possibly a negligible, factor in adults. Pulmonary tuberculosis, which causes nearly 90 per cent. of the deaths from all varieties of tuberculous infection, is practically never due to infection with the bovine bacillus. In children under five years of age, however, bovine infection is a serious matter and is responsible for a large proportion of the rare type of alimentary tract tuberculosis. More than one-half of the cases of cervical adenitis and abdominal tuberculosis are due to bovine infection. Taking the statistics as presented by Park and Krumwiede, it is a legitimate conclusion that in young children the bovine tubercle bacillus causes from 6 to 10 per cent. of the deaths from tuberculosis. The Final Report of the British Royal Commission on Tuberculosis (1911) sums up the results of two years' investigation in the following words: "The evidence which we have accumulated goes to demonstrate that a considerable amount of tuberculosis of childhood is to be ascribed to infection with bacilli of the bovine type transmitted to children in meals consisting largely of the milk of the cow."

Predisposing Factors.—Few diseases are so completely under the sway of predisposing influences as tuberculosis. Modern city life, especially, affords many opportunities for infection, and a large majority of human beings undoubtedly swallow or inhale tubercle bacilli at some time during their existence. It is probable that nearly all adults living in cities become infected to a greater

[1] Park and Krumwiede: Jour. Med. Res., 1910, 23, p. 205; 1911, 24, p. 313.

or less degree. Nägeli,[1] in a comprehensive study of five hundred autopsies upon the bodies of adults dying from all causes, in which the tissues were examined with particular care, found tuberculous lesions in 97 per cent. Infection in early life is probably more common than has been supposed; Harbitz[2] demonstrated latent tubercle bacilli in the lymph-nodes of eighteen children under eleven years old, none of whom showed any evidence of tuberculous lesions. Practically but a small proportion of persons escape infection. It is evidently, therefore, that only when there is a concurrence of favoring factors are tubercle bacilli able to gain a foothold and proliferate to such an extent as to overpower the natural resistance of the organism. Among these predisposing conditions may be mentioned especially the influences of dampness, sedentary life, insufficient or unsuitable food, and the general features of an "indoor" environment. Alcoholism is another very important predisposing factor, although it is often difficult to separate its effects from those of its bed-fellow, poverty. The baneful results of wasting diseases like diabetes, typhoid fever, and whooping-cough are well known to all physicians. The influence of occupation is often marked, certain trades which involve the breathing of dust claiming a disproportionate number of victims from consumption. Thus English statistics show that, taking the death-rate from tuberculosis and other affections of the respiratory system among agriculturists as 100, the rate among potters and workers with earthenware is 453, that of cutlers 407, of plumbers 373, of glassmakers 335, etc.[3] Excessive temperature and moisture have a similar influence, as shown in the disproportionate number of deaths among laundry workers and the operatives in wet spinning-rooms. The effect of mode of life not only upon the inception, but upon the progress, of the disease is well known. It is a commonplace that pulmonary tuberculosis may often be definitely arrested by a change in the manner of living. Wild animals in a state of nature are not naturally liable to the disease, but when kept in confinement in zoölogical gardens often quickly succumb. From a biologic viewpoint tuberculosis is primarily and chiefly a disease of men living in houses and of cattle kept in stables.

[1] Nägeli: Virchow's Arch., 1900, 160, p. 426.
[2] Harbitz: Jour. Infect. Dis., 1905, 2, p. 143.
[3] Newsholme: Vital Statistics, London, 1898, p. 183.

It is reasonable to attribute part of the marked decline in the death-rate from consumption which has occurred in nearly all civilized countries during the last few decades[1] to a widely diffused amelioration in the conditions of life. Better food, better ventilation, shorter hours of work, and a more general education respecting the possibilities of infection are all potent agencies in restricting infection. Newsholme[2] believes that the establishment of special hospitals and retreats for advanced cases has been the main factor in producing the decline in tuberculosis which has taken place in recent years. There is little doubt that from the standpoint of prevention the segregation of the advanced cases of tuberculosis is one of the most valuable means of diminishing tuberculosis dissemination. The care of incipient cases of the disease should not be allowed to overshadow the importance to the community of preventing the broadcast distribution of tubercle bacilli by the advanced cases.

From the foregoing considerations the advantages of compulsory notification of all cases of tuberculosis seem to be manifest. The proper disinfection of the dwelling-places, the protection of the patient and his friends, and the education of the community at large can be accomplished only by this means. The most important sources of infection and the most mischievous predisposing influences can thus be brought under administrative control.

Heredity.—That heredity exercises a marked influence upon tuberculosis has been long an article of popular belief. When examined, however, this belief is seen to rest upon a hardly adequate foundation. Association of parents and children in the ordinary intimate home life presents opportunities so numerous and so favorable for communication of the disease that it is natural enough that the disease should haunt certain families. Family infection may thus simulate inheritance. The problem in each case is to distinguish between environmental and true congenital influences. In connection with hereditary tuberculosis three possibilities present themselves: (1) Inheritance of a special susceptibility, metabolic

[1] In London the tuberculosis death-rate fell from 3.12 per 1000 in 1884 to 2.34 in 1901, to 1.32 in 1910, and to 1.07 in 1920; in Berlin from 3.6 in 1884 to 2.39 in 1902, and 1.48 in 1920; in Vienna from 7.2 in 1884 to 4.76 in 1900 and to 2.74 in 1910; in New York from 4.45 in 1884 to 2.70 in 1903 and to 0.83 in 1923.

[2] Newsholme: Jour. of Hyg., 1906, 6, p. 304.

or structural, to tuberculosis; (2) germinal transmission; (3) placental infection.

(1) Doubt has sometimes been expressed regarding inheritance of a "tendency." Apart from the legacy of a generally feeble constitution which predisposes to tuberculosis as to other diseases, it is thought by some that there is no inherited inborn liability to tuberculous infection. It is difficult, however, to reconcile this view with what is known of racial resistance and susceptibility to specific infection. The familiar biologic facts concerning heredity and variation attest the existence of individual characteristics of all sorts. It is no more improbable that susceptibility to tuberculosis should exist in certain families than that ordinary sheep should be much more susceptible to anthrax than Algerian sheep. Liability to infection with a particular parasite may as conceivably be a congenital character as the color of the hair or eyes or an aptitude for music or mathematics. In the case of tuberculosis this may depend upon the transmission of some structural character, such as a peculiarity of the circulation of the lung apices, or upon more obscure metabolic peculiarities. The eminent biologic statistician, Karl Pearson, as the result of a careful statistical inquiry, has concluded that a consumptive predisposition or diathesis is inherited in the same way and with the same intensity that familiar physical characters are inherited.[1] At the same time it is clear that in any particular case such liability may be difficult to demonstrate, owing—(*a*) to the influence of predisposing causes, (*b*) to the possibility of intrauterine infection, or (*c*) to greater facility of infection from tuberculous relatives.

(2) The likelihood of germinal transmission is exceedingly remote. The ovum is practically never infected.[2] In human semen tubercle bacilli have been found in only a very small proportion of cases, and it does not seem likely that an egg invaded by tubercle bacilli just after fertilization would undergo normal development. In no case has parentally transmitted tuberculosis been traced clearly to the male parent; a tuberculous mother is back of the cases that have been observed (intrauterine infection).

[1] Pearson, Karl: "A First Study of the Statistics of Pulmonary Tuberculosis," London, 1907.

[2] One positive observation by Baumgarten is on record: Arb. a. d. path.-anat. Inst., Tübingen, 1891–92, 1, p. 322.

(3) Intra-uterine or placental infection, although rare, undoubtedly occurs. As many as 70 well-established cases in man have been put on record; the number of observed cases in cattle is several times as great. In identifying cases of true congenital tuberculosis great care has to be taken to eliminate possibilities of extrauterine infection, such as often occur when young are born from a tuberculous mother; numerous animal experiments have resulted in showing that the young of infected mothers are infected, as a rule, only when they are suckled by the tuberculous parent; if transferred to a healthy foster-mother, they remain healthy. On the whole, placental infection is probably an insignificant item in the totality of tuberculosis.

Tuberculin.—The substance originally known as tuberculin[1] (T.O.) is prepared by filtering a glycerol-broth culture of the tubercle bacillus and then concentrating upon the water-bath to about one-tenth its original volume; when stored in a cool dark place, it may retain its properties for months. Many modifications of the original method have been employed. The Bureau of Animal Industry in the United States dilutes with weak carbolic acid the thick syrupy liquid of the concentrate, which in its original condition is difficult to handle in the field. The amount of fluid to be injected for cattle of medium weight is thus increased from 0.25 c.c. to about 2 c.c. Practically all the extracts of the tubercle bacillus contain the poisonous nucleoprotein or its chemical derivatives.

When a small amount of tuberculin is injected into a healthy animal, there is no apparent constitutional disturbance; but when the same quantity is inoculated into an animal with tuberculous lesions, a remarkable selective action appears. There is marked congestion around the tuberculous area, accompanied by necrosis and sloughing off of the tuberculous tissue; fever and other constitutional symptoms also appear.

Tuberculin Reaction.—The tuberculin reaction has been made a cardinal feature in the diagnosis of tuberculosis in cattle, and to some extent in man. Its chief practical application is in detecting the disease in dairy cows. The test on cows is made by injecting subcutaneously from 20 to 40 centigrams of tuberculin and noting any change in the temperature of the suspected animal. The temperature should be taken every two hours on the day prior to the

[1] Koch: Deut. med. Wchnschr., 1890, 16, p. 1029; 1891, 17, pp. 101, 1189.

injection, and at least every two hours on the day of the injection and on the following day. The normal temperature of the cow may vary considerably,[1] and should always first be determined; a rise of from 1.5° to 3° C. warrants the inference that the animals is tuberculous; the tuberculin reaction has been often controlled by autopsy, and for all practical purposes is specific and unequivocal. The information afforded by a positive outcome, as a rule, can be relied on implicitly, but a negative reaction is not always proof of the absence of infection. Failure of the test is most likely to occur in advanced and clinically recognizable cases.

The mechanism of the tuberculin reaction, so far as understood, is as follows: The tuberculin acts as a local specific irritant upon the tuberculous foci, producing intense hyperemia and disintegration of the tuberculous mass. This phenomenon has been interpreted as an acceleration of a process going on more slowly under ordinary conditions. The disintegration is accompanied by the generation or liberation of toxic substances which enter into the general circulation and produce fever and other constitutional disturbances. Insusceptibility to the tuberculin reaction can be produced by repeated doses, a fact that has been taken advantage of by unscrupulous cattle dealers.

In addition to the constitutional changes produced by tuberculin inoculation into a tuberculosis subject, a local reaction also occurs. Von Pirquet[2] found that the application of tuberculin to the abraded skin caused a characteristic local reaction in tuberculous infants, and no reaction or a very slight one in healthy infants. Corresponding with the fact brought out by autopsies, of the all but invariable infection, past or present, of older children and adults, the reaction is usually positive for those over the age of eighteen years. Calmette[3] has made use of this principle in a method sometimes applied for purposes of diagnosis. A solution of tuberculin freed from glycerol by precipitating with alcohol and redissolving in sterile water is instilled into the conjunctival sac. In subjects with active tuberculosis a general congestion of the conjunctiva occurs, and is at its maximum in from six to ten hours, while with the normal individual no reaction takes place. Neither method is used so generally at the present time as the sensitive intracutaneous test

[1] Bull. 7, Bureau of Animal Industry.

[2] Von Pirquet: Berl. klin. Wchnschr., 1907, 44, p. 644.

[3] Calmette: Presse médicale, 1907, 15, Orig., pp. 388, 443.

of Mantoux.[1] This consists in injecting 0.05 c.c. of a 1 : 10,000 dilution of old tuberculin into the superficial layer of the skin. Infiltration and hyperemia about the site of injection denotes a positive reaction.

Long[2] has devised a test of tuberculous infection which is of great value in the experimental study of the disease. It was found that if tuberculin is introduced in a small dose into the testicle of a tuberculous guinea-pig, a prompt reaction occurs with marked hyperemia and swelling and a progressive degeneration of the germ cells. The same amount of tuberculin has no effect, either early or later, upon the testicle of a nontuberculous guinea-pig. Tuberculin prepared from a nonprotein medium elicits a reaction nearly as intense as that caused by protein-rich "old tuberculin." The acid-fast turtle, frog, grass and smegma bacilli appear to contain tuberculin, since their injection causes a definite testicular reaction.

Immunity; Protective and Curative Inoculations.—An animal already carrying tuberculous infection is strongly resistant to a fresh inoculation (Koch). This significant fact has led to much study, and to numerous attempts to produce heightened resistance as a mode of protection against progressive disease.

Animal experiments by Pearson and Gilliland,[3] von Behring, and others have shown that cattle are protected in a marked degree against inoculation with bovine bacilli if they are first injected with the less virulent bacilli of human origin. Vallée[4] found in an extensive series of experiments upon full-grown cattle and calves that if the animals were fed with a highly attenuated strain of the tubercle bacillus (derived from a horse) they acquired temporary immunity, the more complete the younger the animal.

In recent years protective inoculation against tuberculosis has been advocated and to some extent practiced, in three different ways: (*a*) Inoculation with minute doses of living, virulent bacilli. This method, which is similar to the method once used of inoculation with human virus in smallpox, means the production of real if mild infection. However effective as a means of protection, the risk incurred by such a method must always be considerable.

[1] Mantoux: Münch. med. Wchnschr., 1908, 55 II, pp. 2117, 2516.
[2] Long: Amer. Rev. Tuberc., 1927, 9, p. 215.
[3] Pearson and Gilliland: Proc. Path. Soc., Philadelphia, 1904, 6, p. 105.
[4] Vallée: Ann. de l'Inst. Past., 1909, 23, p. 585.

(*b*) Inoculation with attenuated or avirulent bacilli. Modification of tubercle bacilli by contact with chemicals or by long-continued cultivation on special media has been known for years as a means of obtaining a living, relatively harmless vaccine. Calmette and his associates[1] have carried on with great enthusiasm extensive experiments in this field. By the use of successive cultivations on a special potato-bile-glycerol medium living cultures of little or no virulence are obtained. These are designated as BCG vaccine (the bacillus of Calmette and Guérin). On being assured by animal experiments of the harmlessness of the BCG cultures, Calmette and his fellow-workers applied this method to the vaccination of children. Up to the present some thousands of French children have been vaccinated, with results that are distinctly encouraging to those carrying on the work. It is yet too early to pronounce a final judgment as to the success of this method of immunization. It is difficult to maintain close supervision for a sufficiently long period over statistically adequate groups of vaccinated and unvaccinated persons. The steadily widening use of preventive measures is also a complicating factor in any long-time study of this disease. It is not yet certain whether tubercle bacilli once attenuated may not again increase in virulence on prolonged contact with animal tissues.

(*c*) Inoculation with dead bacilli. Tubercle bacilli killed by heat are still capable of conferring some immunity. The degree of immunity obtained by this method does not seem high, but some observers have reported favorable results in children who have been inoculated with dead bacilli and kept under observation for as long as four years. The limited statistical evidence for inoculation with killed cultures seems practically on a par with that for BCG vaccine.

A variety of antibodies can be produced by inoculation with tubercle bacilli and their products. Agglutinins and precipitins are quite uniformly generated. Bacteriolytic substances are possibly formed, but experimental difficulties stand in the way of their ready demonstration. Several observers, notably Maragliano,[2] have reported that the serum of immunized animals possesses neutralizing power for the products of the tubercle bacillus. It is still uncertain how far this power is due to the presence in the

[1] Calmette: "Tubercle Bacillus Infection and Tuberculosis in Man and Animals" (transl.), Baltimore 1923, pp. 689; Ann. de l'Inst. Past., 1927, 41, pp. 201–368.

[2] Maragliano: Berl. klin. Wchnschr., 1904, 41, pp. 603, 643.

immune serum of any substance similar to the true bacterial antitoxins. Practically, the use of antisera in the treatment of tuberculosis has proved of no value.

The presence of specific opsonins in tuberculosis has been utilized in gaging the amount and frequency of the doses of tuberculin. It is thought that by keeping watch of the opsonic index (p. 170) the injection of bacillary extracts and emulsions can be advantageously timed. Trudeau,[1] however, did not consider determination of the opsonic index as necessary to control, and in fact preferred to base the treatment on clinical signs, giving a dose at first far below that expected to excite reaction, and gradually increasing the dose as the condition of the individual patient indicated. Jeans and Sellards[2] also do not consider the opsonic index as sufficiently accurate for the control of tuberculin therapy.

When the "original" or "old" tuberculin ("T. O.") was first introduced by Koch, it was thought to have value as a curative agent, but the sanguine expectations at first aroused were not realized. Tuberculin is probably of value solely in its effect of stimulating focal proliferation. In lupus, a form of skin tuberculosis, good results have frequently been obtained from the sloughing off of the diseased and necrotic tissue, but in other kinds of tuberculosis the results have not been remarkably successful. A "new" tuberculin ("T. R." = tuberculin residuum) was prepared by Koch in 1897[3] by macerating living virulent bacilli and extracting the mass with water, and then making an emulsion of the residuum. Later Koch[4] advocated the use of an emulsion ("B. E." = bacillary emulsion) of the entire substance of pulverized young virulent bacilli in 20 per cent. glycerol. Denys[5] introduced the use of the unaltered filtrate from broth cultures ("B. F." = broth filtrate). Whatever the form of tuberculin employed, the governing principle of the tuberculin treatment would seem to be to avoid from the outset the production of a clinical reaction, and to impart, not too rapidly, a tuberculin immunity which will enable the patient to tolerate quantities much larger than the initial dose. During recent years the use of tuberculin for curative purposes has diminished.

[1] Trudeau: Amer. Jour. Med. Sci., 1907, 133, p. 813.
[2] Jeans and Sellards: Bull. Johns Hopkins Hosp., 1907, 18, p. 232.
[3] Koch: Deut. med. Wchnschr., 1897, 23, p. 209.
[4] Koch: Deut. med. Wchnschr., 1901, 27, p. 829.
[5] Denys: Bull. méd. Paris, 1906, 20, p. 772.

Other Acid-fast Bacteria.—The once current view that the tubercle bacillus was unique in its resistance to decolorization by acid has been modified by the discovery of a considerable number of micro-organisms possessed of the same characteristic. Some of these, such as the bacillus of leprosy, are closely related biologically to the tubercle bacillus. B. lepræ, however, differs somewhat in form from the tubercle bacillus, is stained with less difficulty, and, owing to the relative infrequency of leprosy and its occurrence in characteristic lepra cells, its resemblance is not likely to engender confusion in matters of practical diagnosis. The smegma bacillus, on the other hand, which is found in the preputial secretion and between the labial folds of the vulva, is often difficult to distinguish from the tubercle bacillus. Its occurrence in the feces and urine, where it has been mistaken for the tubercle bacillus, is said to have sometimes led to serious diagnostic error—even to unnecessary kidney extirpation. In collecting samples of urine, contamination with smegma bacilli may be avoided to some extent by catheterization. The smegma bacillus differs very little, if at all, in morphology from the tubercle bacillus, but is said by some observers to be decolorized, as a rule, by treatment with simple alcohol; it is also said to be shorter and to show minor differences that are likely to be noted by the experienced observer. Such distinctions cannot be safely depended upon, and in doubtful cases guinea-pig inoculations should be made.

Acid-fast bacilli have been found in the turtle and the frog.

A number of other acid-fast bacteria have been isolated from such substances as butter, hay, and dung. These organisms, of which about forty varieties have been described, grow readily at a low temperature upon the ordinary culture-media, often with production of a brownish pigment. They seem to be widely distributed as saprophytes in nature. They have also been observed in sputum in some cases in which the diagnosis of tuberculosis could be excluded on clinical and anatomic grounds. Several observers have found these or similar organisms in man in connection with pathologic conditions, such as bronchitis (Marzinowsky[1]) and pulmonary gangrene (Fränkel,[2] Rabinowitsch[3]). Animal inoculation

[1] Marzinowsky: Centralbl. f. Bakt., 1900, 28, p. 39.
[2] Fränkel: Berl. klin. Wchnschr., 1898, 35, pp. 246, 880.
[3] Rabinowitsch: Deut. med. Wchnschr., 1900, 26, p. 257.

does not always surely differentiate these organisms from the tubercle bacillus, since some varieties produce histologic changes closely simulating those of true tubercle formation. The rapid growth of the "grass" and "butter" bacilli in artificial media, in most cases at about 20° C., is the principal differential feature. It is evident that observers may readily fall into error by endeavoring to discover by microscopic examination alone the presence of the tubercle bacillus in such substances as butter or milk. Even the peritoneal inoculation of butter containing the ordinary "butter bacilli" is likely to result in lesions that closely resemble those of tuberculosis. Many of the reported findings of tubercle bacilli in dairy products are therefore of somewhat doubtful value.

Acid-fast saprophytes appear to differ serologically from all other acid-fast bacilli.[1]

[1] Furth: Jour. Immunol., 1926, 12, p. 273.

CHAPTER XXIII

THE BACILLUS OF LEPROSY (BACILLUS LEPRÆ)

Rogers estimated that in 1924 the world contained about 3,000,000 lepers. At the present day this disease is most common in India (100,000 cases), Japan (100,000), and other Asiatic countries. Approximately 1200 cases were thought to exist in the United States in 1926.

In 1848 Danielssen[1] recognized that certain peculiar cells which were found in leprous tissue were characteristic of leprosy, and as early as 1872 Armauer Hansen[2] announced his discovery of small rods lying within the "lepra cells." The application of staining methods by Neisser and Hansen[3] showed these rods to be bacilli; Hansen's discovery of the bacteria in leprosy, therefore, ranks as one of the earliest observations of pathogenic bacteria.

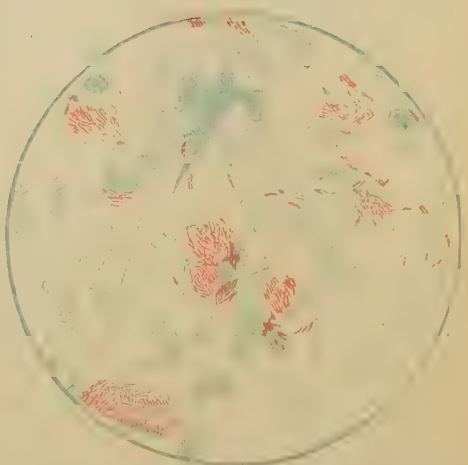


Fig. 104.—Bacillus lepræ. Smear preparation from nasal mucosa (Hansen: Kolle and Wassermann).

Characteristics of the Leprosy Bacillus (B. lepræ). Morphologically the leprosy bacilli resemble closely the tubercle bacilli. They are long (6 μ), slender rods, usually straight, but sometimes slightly curved. They have no power of independent movement, and are not known to produce spores. The bacilli are sometimes seen lying free in the lymphatic spaces, but the great majority are

[1] Danielssen and Boeck: "Traité de la spédalskhed," Tr., Paris, 1848.

[2] Hansen, Armauer: Norsk Mag. f. Laegevidensk., 1872, 2, p. 1.

[3] Neisser and Hansen: Breslauer ärzt. Ztschr., 1879, No. 20; Archiv f. path. Anat., 1880, 79, p. 32.

ensconced in the cells. Their arrangement in the cells is characteristic, several bacilli being usually grouped together in bundles like packets of cigarettes (Fig. 104).

The staining reaction of these organisms is much like that of the tubercle bacilli. They stain somewhat more readily than the latter, and also decolorize more quickly with acids, but the difference is not sufficient to serve for differential diagnosis. The presence of large numbers of bacilli within the cells, together with the clinical features, make it possible ordinarily to distinguish leprosy from tuberculosis without difficulty. Sections of tissue may be left twenty-four hours in carbol-fuchsin, then decolorized with hydrochloric acid and alcohol, and finally counterstained with aqueous methylene-blue.

Cultivation.—Numerous unsuccessful attempts to cultivate the leprosy bacillus on artificial media were made for years by bacteriologists in all lands. Kedrowski's work may be particularly mentioned.[1] This investigator cultivated an organism from leprous tissues by use of an agar medium prepared with expressed juice from the human placenta. As described by him the organism so cultivated was highly pleomorphic, with branched, acid-proof, and non-acid-proof stages. His description suggests an original mixed culture from which one or more forms were later eliminated by animal passage. Clegg[2] was the first to obtain apparent growth and continued multiplication in subculture upon an artificial medium. The method employed consisted in inoculating emulsions of leprous tissue upon a medium where amebæ and bacteria were growing symbiotically. By subsequent heating at 60° C. for thirty minutes the amebæ and symbiotic bacteria were destroyed, while the more resistant B. lepræ survived in pure culture.

Clegg's results were soon confirmed by others, including Duval,[3] who succeeded in growing an organism regarded as B. lepræ directly from human tissue upon a medium without living organisms.

The success of both Clegg's and Duval's methods is thought to depend upon the presence of substances arising from the digestion of nucleoproteins. These substances may be added directly (tryptophane, Duval), or be produced in the medium by trypsin

[1] Kedrowski: Ztschr. f. Hyg., 1901, 37, p. 52.
[2] Clegg: Philippine Jour. of Science, 1909, 4, p. 403.
[3] Duval: Jour. Exper. Med., 1910, 12, p. 649.

digestion (Duval), or by bacterial action (Clegg[1]). Apparently, therefore, B. lepræ is unable to split or hydrolyze native albumin, but must depend upon amino-acids ready formed by other bacteria or by tryptic enzymes. When bits of infected tissue are transferred to ordinary laboratory media, multiplication of the leprosy bacilli generally takes place, owing to the hydrolytic action of the associated bacteria.

Duval gives the following description of the method of cultivation used by him: "The most efficient method for obtaining the initial growth of B. lepræ is to transfer bits of the leprous nodule to slanted 1 per cent. alkaline nutrient agar and seed with some one of the proteolytic non-spore-bearing bacteria, which in the course of ten days to two weeks at 37° C. digest the protein sufficiently to cause the contained Hansen rods to multiply. It is essential to have the medium alkaline in order to inhibit too profuse a growth of the hydrolizing organism. In the softened tissue the Hansen bacilli increase steadily and continue to do so in transplants to other media as long as the dissociate products of the host tissue last. The organism used to hydrolize the tissue is subsequently eliminated by heating the cultures at 60° C. for thirty minutes (Clegg's method), which does not affect the viability of the Hansen rods. While multiplication takes place readily in the digested host-tissue, growth ceases upon other nutrients unless there are added the intermediate products of protein digestion, such as the filtered autolized liver, blood-serum, placenta, etc."[2] At present it seems reasonably certain that some of the chromogenic acid-fast cultures isolated from leprous nodules are not to be regarded as the real micro-organisms of leprosy, but are rather to be classed with such organisms as the smegma bacillus and grass bacillus. This is particularly

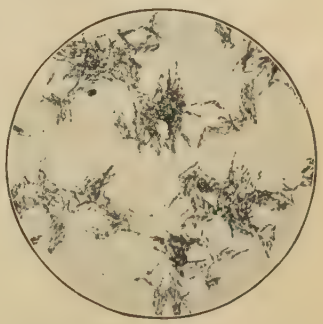


Fig. 105.—Pure culture of B. lepræ, showing the characteristic morphology and arrangement of the bacilli (Duval).

[1] According to Duval the amebæ used in Clegg's method are entirely unnecessary, the essential changes in the culture medium being brought about by the bacteria, many species, such as Bacillus typhosus, B. prodigiosus, and B. pyocyaneus, being suitable for this purpose.

[2] Duval: Jour. Med. Res., 1913, 28, p. 165.

true of certain freely growing strains. It is still uncertain whether any of the genuine leprosy bacillus strains are chromogenic. Diphtheroid forms sometimes observed are considered by some authors to be involution forms. Filamentous, non-acid-fast organisms isolated from leprous nodules are probably to be looked upon as associated organisms not directly concerned with the leprous process. The micro-organism of leprosy seems to be acid-fast, slow-growing even under the most favorable conditions, and probably in most instances, if not all, non-chromogenic.

Animal Experiments.—Great difficulty has been experienced by investigators in producing any multiplication of B. lepræ in the tissues of the lower animals. Nicolle[1] was one of the first to report the development of typical leprous nodules in the monkey following subcutaneous inoculation of bits of leprous tissue. Clegg,[2] Sugai,[3] and Duval[4] have observed the development of leprous lesions in such animals as guinea-pigs and Japanese dancing mice. Duval has infected Japanese dancing mice, guinea-pigs, and monkeys (Macacus rhesus) by the use of pure cultures isolated by him. In the monkey, according to Duval,[5] disseminated leprosy has followed the repeated injections of large quantities of pure cultures. The infected animals are said to present the typical clinical picture of human leprosy.

Duval and Gurd[6] state that: "Two factors are of great importance in effecting infection. In the first place, a sufficiently large number of organisms must be employed, and, what is still more important, second and subsequent inoculations are more liable to produce leprous lesions than are primary injections." The interpretation of this apparently increased susceptibility following a preliminary dose is still uncertain.

Duval's work indicates that the leprosy micro-organism gives specific immunity reactions (agglutination and bacteriolysis). Inoculation experiments are of little value since indentical lesions seem to be produced in experimental animals by the leprosy bacilli and the familiar acid-fast bacilli from hay, butter, and smegma.

[1] Nicolle: Sem. méd., 1905, 25, p. 116.
[2] Clegg: Philippine Jour. of Sci., 1909, 4, p. 403.
[3] Sugai: "Lepra," 1909, 8, p. 203.
[4] Duval: Jour. Exper. Med., 1910, 12, p. 649.
[5] Duval: Jour. Exper. Med., 1911, 13, p. 374; 1912, 15, p. 292.
[6] Duval and Gurd: Jour. Exper. Med., 1911, 13, p. 181.

Bayon's comparative experimental study of the leprosy cultures of Clegg, Duval, Kedrowski, and others showed that only certain strains (Kedrowski's) produced leprous lesions on injection into animals.[1] A very comprehensive review of the whole puzzling question of the bacteriology of leprosy has been given by Wolbach and Honeij.[2] No final conclusion on the matter seems possible at present.

Rat Leprosy.—Stefansky[3] was the first to describe a leprosy-like disease in rats. His observations have been confirmed by a number of workers in regions where human leprosy is prevalent. Wherry[4] and others have observed this disease in rats caught in the neighborhood of San Francisco. A rat in the advanced stage of this disease presents a clinical picture closely resembling that of human leprosy. Currie and Hollmann[5] were able to produce the disease by inoculation in white rats. They also found that certain mites abundant on the rats during the illness of these animals contained the bacilli of rat leprosy in considerable numbers in their digestive tracts, and they infer that these parasites might conceivably be a means of transmitting the disease. Wherry[6] had previously found the bacilli in the bodies of rat lice. Schmitt,[7] in examining the relation between rat and human leprosy, found that the complement fixation reaction (p. 177) showed complete fixation in mixtures of sera from cases of human leprosy of the various types with antigen prepared from the lesions of leprosy in rats.

Pathogenicity for Man.—Although any organ or tissue may be attacked with varying results, two distinct types of leprosy are usually recognized—the nodular and the anesthetic. The former, which is the more acute, is characterized by the development of masses of granulation tissue, the so-called leproma, which may appear superficially in different parts of the body, and by their growth and coalescence cause terrible distortion and mutilation. The anesthetic type, or nerve leprosy, progresses more slowly than

[1] Bayon: Brit. Med. Jour., 1912, II, p. 1191.
[2] Wolbach and Honeij: Jour. Med. Res., 1914, 24, p. 367.
[3] Stefansky: Centralbl. f. Bakt. I, Orig., 1903, 33, p. 481.
[4] Wherry: Jour. Amer. Med. Assoc., 1908, 50, p. 1903.
[5] Currie and Hollmann: Public Health Bulletin, No. 41, 1910.
[6] Wherry: Jour. Infect. Dis., 1909, 6, p. 630.
[7] Schmitt: Univ. of Calif. Publication in Pathol., 1911, 2, p. 29.

the other form, the average duration of the cases being nearly twice as long (eighteen years), some being known to extend over thirty-five or forty years; atrophy of the muscles and other trophic disturbances accompany the nerve lesions.

In both forms of leprosy the Hansen bacillus is found in all cases: in enormous numbers, as a rule, in the lesions of nodular leprosy; less abundantly in the anesthetic type. As already stated, it is the prevailing opinion among students of this disease that while a few bacilli occur free in the lymphatic spaces, the great majority are contained within the cells.[1] The nucleus itself is not invaded. Unna[2] maintains that they lie exclusively in the lymph-spaces; other observers (Leloir[3]) believe that they occur partly in one, partly in the other, situation. Almost any organ or tissue may be the site of a leprous growth. Bacilli have been found in practically all parts of the body. The kidneys are usually invaded, the liver and spleen always. The bacilli have been seen by several observers in the cells of the central nervous system; they are sometimes encountered in the blood, generally in the leukocytes, but occasionally free (Hansen).

Evidence of the direct inoculability of leprosy from man to man is quite inadequate. Many attempts to infect healthy persons have been made and have failed, and one often-cited instance of successful inoculation is by no means unimpeachable. In the case of the criminal Keanu in the Hawaiian Islands, reported by Arning,[4] implantation of material from a leprosy nodule was followed by the development of true leprosy, which terminated fatally six years after inoculation. The experiment, however, did not exclude the important source of error involved in the facts that Keanu was a native of a country in which leprosy was common, that he had lived among lepers, and that members of his family were lepers.

The indirect evidence of transmission is more significant. Manson[5] cites the case of a leper, an Irishman who acquired his disease in the West Indies. On his return to Ireland his bed was shared

[1] Hansen is of opinion that all the bacilli are in the cells originally and only appear in the lymph-spaces when the normal relations are disturbed.

[2] Unna: Deut. med. Wchnschr., 1886, 12, p. 123.

[3] Leloir: Comp. rend. Acad. Sci., 1885, 101, p. 97.

[4] Arning: Archiv. f. path. Anat., 1893, 134, p. 319.

[5] Manson: "Tropical Diseases," London, 1900, p. 448.

by his brother, who, moreover, sometimes wore the leper's clothes. The brother, who had never been in any foreign country, became, in time, an undoubted leper. In this case communication from one person to another was practically demonstrated.

Currie[1] found that a large percentage of cases studied in Hawaii gave a history of exposure, and that usually such exposure was of an intimate character. The importance of environment as contrasted with heredity is also emphasized by Hollman.[2]

Mode of Transmission.—The numerous cases in which healthy persons, such as asylum attendants, have been more or less in contact with lepers for long periods without contracting the disease have induced some observers to deny the possibility of contagion. Another explanation of this freedom from contact infection, and one more in accordance with other observations, can, however, be advanced. This is that the conditions necessary for successful infection are rarely met with, and that consequently infection does not take place simply by association of a leprous with a sound individual. The conditions that render transmission of the disease possible are entirely unknown; they may concern the virulence of the infecting bacillus, the availability of a suitable portal of entry, or some peculiar and rarely occurring state of receptivity on the part of the individual attacked. The cardinal point in the epidemiology of leprosy appears to be that intimate contact with a leprous individual or residence in a locality where leprosy is endemic is a necessary condition of infection. There is no valid evidence of the occurrence of the bacillus of leprosy outside of the human body, and in view of all the facts there seems no escape from the doctrine of direct contagion.

One way in which the bacillus may leave the body is in the nasal mucus. Sticker[3] and other observers have found bacilli in the secretions of the nose in a large proportion of cases. Bacilli may sometimes be discharged from the mouth or nose in small particles of mucus driven out by violent coughing or sneezing. In the opinion of many writers, the mucous membrane of the nasopharynx is the point at which the bacteria are introduced into the body, as well as the chief source from which infection is spread.

[1] Currie: Public Health Bull., No. 41, Washington, Nov., 1910.
[2] Hollman: Public Health Bull., No. 39, Washington, Sept., 1910.
[3] Sticker: Deut. med. Wchnschr., 1897, 23, p. 219.

Currie's observations[1] indicate the possible transmission of the disease by means of flies, since these insects when fed upon leprous fluids contain the bacilli in their intestinal tracts for several days.

Much light is thrown on the contagious character of leprosy by the success that has attended the isolation and segregation of leprous patients. The experience of Norway showed that a careful but not unduly rigorous system of separation was accompanied by a diminution of the number of cases from 2870 in 1856 to 577 in 1900. The circumstance that infection does not invariably follow chance contact or association should not, therefore, lead to neglect of the facts that leprosy is a bacterial disease; that up to the present the specific germ has not been found under natural conditions, except in the human body; and that, so far as is known, the leper himself is the only means by which leprosy spreads.

From the fact that a number of cases of leprosy often occur in the same family the disease has been regarded by some as "hereditary." The question of the "inheritance" of a bacterial disease has already been discussed in connection with tuberculosis, and the argument as regards leprosy is very similar. Germinal infection (of the ovum or sperm), if it occurs at all, is probably very rare, most lepers becoming sterile early in the disease. Intrauterine infection, although it has been reported, is not common. Hansen has called attention to the remarkable fact that none of the children of one hundred and seventy Norwegian lepers who have from time to time migrated to North America have become diseased. It seems reasonable to suppose, however, that a special tendency to contract leprosy may be inherited, just as any other bodily peculiarity.

At the present time leprosy is most common in countries with moist tropical climates and is very rare in the drier parts of the tropics and in temperate climates.

[1] Currie: Public Health Bull., No. 39, Washington, Sept., 1910.

CHAPTER XXIV

THE GLANDERS BACILLUS (BACILLUS MALLEI)

Glanders is a disease seen, as a rule, only in the solipeds (horse, mule, ass), but occasionally transmitted to other domestic animals, to wild animals, and to man. As in so many other diseases, the early history of this malady is marked by lively oscillations of opinion as to its infectious nature. The doctrine of the spontaneity and non-infectivity of glanders received strong support as late as 1830–40, especially from the famous Alport school of veterinarians in France. In practice the prevalence of this view was attended with disastrous consequences. In 1837 Rayer[1] demonstrated that the horse could be infected by inoculating it with material derived from a case of glanders in a human subject. Owing to the high reputation of the author this experiment made a deep impression, and although a similar experiment had been successfully performed by others prior to this time, Rayer's work may be said to mark the downfall of the dogma of spontaneity.

The specific germ of glanders was discovered in 1882 by Löffler and Schütz,[2] whose results were soon confirmed and extended by Kitt,[3] Weichselbaum,[4] and others.

Morphologic and Cultural Characters.—The glanders bacillus, or B. mallei, is a small rod, straight or slightly curved, usually with rounded ends, and often of irregular contour. It is about the same length as the tubercle bacillus, but is shorter and thicker than the latter (Fig. 106). Rather wide variations in size are observed; the following dimensions may be taken as the average range: length, 2 to 5 μ; breadth, 0.5 to 1 μ. Power of independent movement is absent. The organism is not known to form spores, although there has been much discussion concerning the nature of certain granules and irregularly staining portions of the cell protoplasm. It is now generally admitted that, whatever may be the

[1] Rayer: Mém. Acad. de méd. Paris, 1837, 6, p. 625.
[2] Löffler and Schütz: Deutsch. med. Wchnschr., 1883, 9, p. 197.
[3] Kitt: Jahresber. d. k. Centralbl. Tierärzt-Sch., München, 1883–4.
[4] Weichselbaum: Wien. med. Wchnschr., 1885, 35, p. 665.

physiologic significance of these unevenly stained elements, they are not to be regarded as true spores. No increased power of resistance is shown by cultures containing the granules or coccus-like bodies. In cultures the bacilli frequently occur in pairs, sometimes in short chains. Long filaments with swollen ends and true branching have been seen by several observers and have induced some writers to rank the glanders bacillus with the trichomycetes. (Compare the tubercle bacilli, p. 428.)

B. mallei stains with the ordinary aqueous anilin dyes, although not very readily. The best results are obtained with stains containing alkali, or a mordant such as carbolic acid (for example, Löffler's alkaline methylene-blue, Ziehl's carbol-fuchsin, etc.). Decolorization takes place easily on the application of alcohol or dilute acid; the color is lost also by Gram's method.

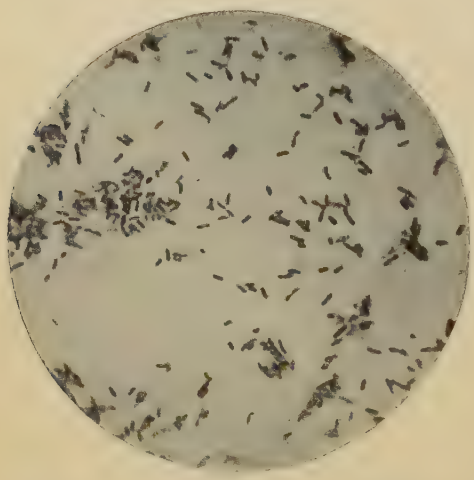


Fig. 106.—Bacillus mallei. Pure culture from glucose-agar. Carbol-fuchsin; × 1200 (Hicks).

Growth occurs on the ordinary media, but is materially aided by the presence of glycerol. Temperatures below 22° C. are unfavorable, the optimum being about 37° C. A slightly acid reaction of the medium is rather favorable than otherwise. Save on potato, the growth presents little that is characteristic. On this medium at 37° C. a tenacious, transparent, honey-like layer is formed, which in time takes on a deeper brownish hue. Often, but not invariably, the potato around the growth becomes tinged a greenish yellow, not unlike the decoloration produced by some cultures of B. pyocyaneus.

In nutrient broth a uniform turbidity is produced, and a white, heavy, viscous sediment collects at the bottom of the tube. On agar and glycerol-agar a whitish, translucent streak is formed, with crenated edges, and possessed of a tenacious consistency. Gelatin

is not liquefied. Milk is slowly curdled with acid production by some cultures, but the statements of different observers are not in accord respecting the action on this medium; different cultures doubtless behave differently (Wherry[1]).

As in the case of many other parasitic bacteria, growth does not always take place readily when the glanders bacillus is first transplanted from the animal body to artificial culture-media. After a few transfers, however, the saprophytic habit is firmly established, and by reinoculating at suitable intervals upon glycerol-agar of a uniform reaction (Wherry) and keeping at a low temperature little difficulty is experienced in maintaining living cultures.

Toward physical and chemical agents B. mallei manifests slight resistance, being readily destroyed by heat and antiseptics. Desiccation experiments have not given uniform results, the reported longevity under drying ranging from a few days to several months. In this particular, pure cultures are said to be more resistant than the bacilli in the nasal secretions from diseased animals (Löffler,[2] Nowikoff[3]).

Pathogenicity for the Lower Animals.—Under natural conditions the horse chiefly is found affected, but cases are occasionally observed in the carnivora (cats, dogs, menagerie animals) and in goats and sheep. Swine and pigeons are slightly susceptible. Cattle and the house-rat are immune. Rabbits and guinea-pigs are susceptible to inoculation.

Glanders manifests itself in an acute and a chronic form. The acute form is ushered in usually by a chill and the appearance of a high temperature in advance of any local manifestation. In a few days the mucous membrane of the nose is inflamed and becomes studded with nodules, the lymphatic system becomes largely implicated, and edematous swellings appear in various parts of the body. General symptoms become more grave, and death follows in from eight to thirty days (Nocard[4]). The mule, and especially the ass, suffer commonly from the acute disease. The chronic form is the more usual type in the horse (90 per cent of cases). A great variety of symptoms and lesions have been noted in the latter

[1] Wherry: No. 24, Publications of Government Laboratories, Manila, November, 1904, p. 24.

[2] Löffler: Arb. a. d. k. Gesund., 1886, 1, p. 141.

[3] Nowikoff: Arch. d. Sci. vét., 1895.

[4] Nocard: "Les maladies microbiennes des animaux," 2d ed., Paris, 1898.

animal, and the disease pursues most diverse courses in different individuals. The nasal membrane is often affected, and there is a profuse and infectious catarrhal discharge. Cutaneous glanders is known by veterinarians as *farcy*, the thickenings of the superficial lymphatics being termed "farcy buds" or farcy "pipes." In all forms of glanders there is a tendency to the production of nodules, which soften and pass over into ulcers.

The glanders nodule has been considered by some writers to be structurally similar to the nodule formed by the tubercle bacillus (p. 435), but most observers are agreed that the former is a degenerative rather than a proliferative formation, and that it is radically different from the tubercle. The acute and chronic types run into one another, the latter frequently terminating in an acute attack. Experimental inoculation with pure cultures has given positive results, not only in the horse, in which the characteristic features of the disease are reproduced, but in guinea-pigs, field mice, and other small rodents. House-mice and white mice show a high but not absolute resistance, in contrast to the great susceptibility of field-mice. The guinea-pig responds to inoculation in a typical fashion, and this fact has been utilized in effecting the differential diagnosis of glanders (see p. 470). Both in the natural and in the experimental infection the bacteria are found chiefly in the nasal secretions and in the contents of the young nodules; in the older ulcers they are relatively few in number. The blood, as a rule, contains glanders bacilli only in acute general infection.

Pathogenicity for Man.—Veterinarians and others having to do with the care of horses are the most liable to contract glanders. Freshly isolated cultures are highly virulent, and a number of fatal infections have occurred among laboratory workers. The acute form of the malady is the more common in man: most cases terminate fatally within two to three weeks, sometimes within a few days of their inception. As in the horse, the mucous membrane of the nostrils, although not invariably affected, is a place of predilection for the glanders nodules and ulcers. Occasionally the chronic form may appear and linger for months or even years, with spreading ulceration and other features closely resembling those observed in the horse (Fig. 107). Recovery from chronic glanders may take place, or the disease may pass into the acute stage.

Path of Entrance.—The avenue by which the glanders bacillus usually enters the body of the horse has not been clearly determined. The intact skin probably rarely, if ever, permits entrance, but a slight wound or injury offers a ready portal, as attested by experimentation. The mucous membrane of the nose, especially if slightly abraded, may become the portal of entry, as may the intact conjunctiva, which, as shown by Conte,[1] can be infected by contact with infectious material in two to four hours, sometimes in thirty minutes. Infection by inhalation must be rare, to judge from

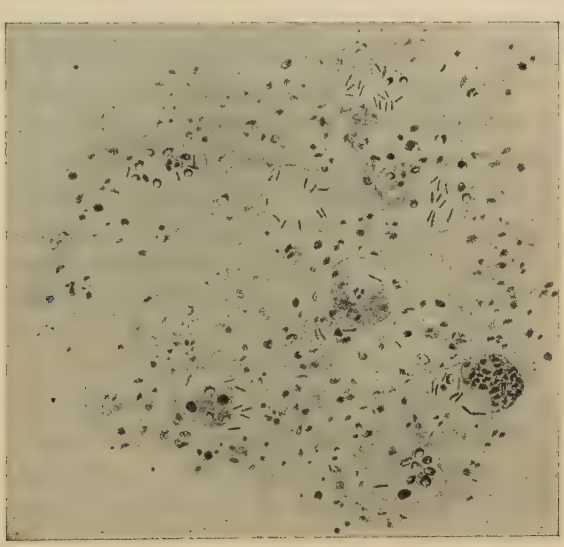


Fig. 107.—Bacillus mallei in giant-cells and exudate of human lungs (Coleman and Ewing).

animal experiments, if, indeed, it ever occurs. According to Nocard, who made a special study of the mode of infection, penetration takes place by way of the alimentary tract in the great majority of cases. There is weighty experimental and other evidence in support of this view.

In man the alimentary tract is certainly not the ordinary channel of entrance; cases are on record of the ingestion of meat from glandered animals without resulting infection. Inhalation likewise hardly enters into consideration. Probably infection through a scratch or other break in the skin is the usual origin of human cases.

[1] Conte: Revue Vétérin., 1893, p. 568.

Diagnosis.—In prebacteriologic days chronic glanders in the horse was frequently separated from other diseases only with difficulty and a considerable measure of uncertainty. At present the diagnosis of glanders is greatly facilitated by: (1) guinea-pig inoculation; (2) the mallein test—*a*, subcutaneous; *b*, ophthalmic; (3) the agglutination method; (4) the complement-fixation test.

1. *Guinea-pig Inoculation.*—A male guinea-pig is injected intraperitoneally with fragments of diseased tissue, scrapings from ulcers, or some of the nasal discharge from a suspected animal. A positive reaction is shown by the testicles becoming red and swollen, usually on the second or third day (Straus[1]). Together with the orchitis (inflammation of the parenchyma of the testicle) there are severe general symptoms which generally culminate in twelve to fifteen days. Grayish nodules are usually found in the spleen and other internal organs. The test is not absolutely specific, for Kutscher[2] and Nocard have shown that an analogous orchitis may be produced by other organisms besides the glanders bacillus. It is often, however, of value, especially when, for one reason or another, other tests are inapplicable.

2. *The Mallein Test.*—Mallein is the concentrated glycerol broth in which the glanders bacillus has grown; it is prepared in the same manner as tuberculin. The mallein reaction consists in a rise of temperature, accompanied by a pronounced local reaction, and in many cases, although not invariably, by more or less profound constitutional disturbances.

(*a*) Subcutaneous injection of the mallein (the size of the dose varying according to the concentration) into a glandered horse is followed by the signs above noted, while in an animal not infected with glanders the temperature is slightly or not at all affected and the general symptoms are absent. The temperature of the suspected animal should be taken at two-hour intervals before the injection is made, and, after the injection, at the ninth, twelfth, fifteenth, and eighteenth hours, at least. The increase of temperature in glandered horses varies from 1.5° to 2.5° C. above the normal, and is distinctly high on the second day after injection. Healthy horses often show a distinct temperature increase on the first day after

[1] Straus: Archives de méd. expér., 1889, 1, p. 489; Comp. rend. Acad. Sci., 1889, 108, p. 530.

[2] Kutscher: Ztschr. f. Hyg., 1896, 21, p. 156.

inoculation, but, as a rule, this disappears quickly. In the use of mallein, as in the tuberculin test, care must be taken to exclude other influences that disturb the normal temperature relations. Experienced observers lay much stress upon the appearance of swelling at the seat of inoculation. In a glandered animal the tumefaction is large, hot, and painful; it increases in size up to twenty-nine to thirty-six hours, persists for about a week, and gradually disappears. In a healthy animal a swelling may occur, but it is never large and vanishes within twenty-four hours.

There is complete agreement among veterinarians regarding the high diagnostic value of mallein. The reaction is specific, is usually sharp and decisive in character, and almost never fails to reveal the presence of infection. Nocard expressed himself very emphatically: "A complete mallein reaction is unequivocal; the animal that reacts is glandered. An animal which does not react to an injection of mallein is not glandered, whatever the character of the symptoms." Recent workers agree, however, that the subcutaneous mallein test is not so reliable as at first supposed, and prefer the ophthalmic test.

(*b*) The ophthalmic test consists in the introduction of the mallein, preferably in tablet form, into the conjunctival sac. The reaction is very reliable and the test can be readily made by every practising veterinarian.

3. The *agglutination method* (Macfadyen[1]) has been extensively used officially in Prussia and Austria. The serum of normal horses agglutinates in dilutions of from 1:200 to 1:300, and the reaction is specific only when rather high dilutions (1:500 to 1:3200) are used; the serum from sound animals, however, sometimes agglutinates the glanders bacillus in a dilution as high as 1:500. Occasionally the reaction fails to appear in the serum of glandered animals. The test is liable to the usual difficulties and sources of error in the hands of an unskilled observer (p. 180). Moore[2] uses a suspension of B. mallei in carbolized salt solution prepared from a glycerol-agar culture, killed by heating to 60° C. for two hours.

4. The *complement-fixation test* (p. 177) is very accurate, but demands special laboratory facilities and is less easy to apply in a practical way than the ophthalmic test.

[1] Macfadyen: Jour. Comp. Path. and Therap., 1896, 9, p. 322.
[2] Moore: Jour. Infect. Dis., 1907, Suppl. No. 3, p. 85.

Immunity.—Permanent immunity to glanders can neither be conferred by an attack of the disease nor produced by any artificial means. Nocard fed with infectious matter three horses which had previously recovered from the disease, and found that these animals showed no resistance superior to that of a healthy control animal. Chronic glanders may exist for years, and is in no wise a warranty against the sudden development of an acute attack.

No very potent or characteristic toxic substance has been obtained from cultures of the glanders bacillus, and attempts at immunization with the products of this organism have been eminently unsuccessful. It is stated by a number of observers that repeated injections of mallein will exercise a curative action upon certain forms of recent infection, but mallein is without immunizing power. The serum of animals treated with mallein injections and the serum of naturally immune animals, such as cattle, are, according to most observers, totally devoid of any preventive or curative value.

The most that has been accomplished in the direction of immunization is a very moderate augmentation of resistance in dogs injected with small non-fatal amounts of living cultures (Straus).

Melioidosis, a disease somewhat similar to glanders, is caused by an organism (Bact. whitmori) closely resembling B. mallei. It has been observed in Rangoon where it is thought to be primarily a disease of the wild rat, communicable to laboratory animals and occasionally to man.[1]

[1] Stanton and Fletcher: Bull. 5, Inst. Med. Res. Federated Malay States, 1924.

CHAPTER XXV

OTHER PATHOGENIC BACILLI

Bacillus (Pseudomonas) pyocyaneus

The blue or blue-green stains that sometimes appear upon surgical dressings long ago attracted the attention of observers, and even before the cause of the phenomenon had been discovered, Fordos[1] carried out some important investigations upon the nature of the coloring substance. In 1882 Gessard[2] proved that the pigment was the product of a specific micro-organism, B. pyocyaneus, which he was able to isolate in pure culture.

Morphology and Cultural Characters.—The cells of B. pyocyaneus vary considerably in size and proportion, but appear usually as small, slender rods, frequently united in pairs and short chains (Fig. 108). A single flagellum is attached to one end, and the organism is actively motile. Spores have never been observed. Gram's stain is negative.

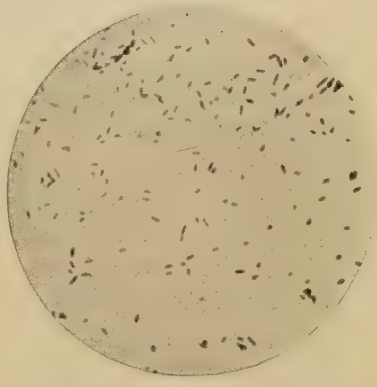


Fig. 108.—Bacillus pyocyaneus. Pure culture on agar. Fuchsin stain. Zettnow prep. (Kolle and Wassermann).

B. pyocyaneus grows readily on all the ordinary culture-media. Gelatin is quickly liquefied, and freshly isolated cultures impart to this medium the characteristic blue-green coloration. Upon agar a yellowish-white surface growth usually develops, the agar itself being richly colored. Broth is rendered turbid, a heavy flocculent sediment is formed, and a tenacious surface pellicle. Indol is produced. Growth on potato is generally luxuriant and of a brownish color; the potato itself is tinged green by freshly isolated strains. Milk is quickly

[1] Fordos: Comp. rend. Acad. d. Sci., 1860, 51, p. 215.

[2] Gessard: La pyocyanine, Thèse, Paris, 1882.

curdled and shows an alkaline reaction; in old cultures the casein is digested.

Products of Growth.—Those cultures of B. pyocyaneus that have been recently isolated from the animal body generate two pigments: a fluorescent green pigment, apparently identical with that produced by a number of common water bacteria (B. fluorescens liquefaciens *et al.*), and a more characteristic deep blue pigment, known as pyocyanin, which can be extracted from solution by chloroform. Ledderhose[1] ascribes to pyocyanin the formula $C_{14}H_4N_2O$. The pigment has no poisonous qualities, as shown by animal experiments. Pyocyanin oxidizes to a dark brown or even black pigment in old cultures, and in strains that have long been under cultivation the brownish color appears without previous development of the blue color. The conditions that determine both the formation of the fluorescent pigment and of the pyocyanin have been exhaustively studied.[2] Oxygen is essential. Sulfate and phosphate are necessary to the formation of the fluorescent pigment, but small quantities of pyocyanin may be formed in the absence of these salts. The enzymes secreted by B. pyocyaneus have also often been the object of special investigation. The sterile filtrates of this organism dissolve gelatin and fibrin; Breymann[3] found an enzyme that coagulated and peptonized milk and was closely bound to the bodies of the bacilli; Emmerich and Löw[4] carried out a long series of observations on a thermo-stabile substance called by them pyocyanase, to which they attributed great importance in immunizing against and in curing certain infections. The results of these latter writers have not been confirmed. The filtrates of B. pyocyaneus likewise possess hemolytic power, but this property seems to be due simply to the alkalinity of the filtrate and not to the presence of any specific "pyocyanolysin" (Jordan[5]).

Broth cultures of B. pyocyaneus are toxic especially for guinea-pigs. According to Wassermann, this toxicity is due, not to an endotoxin, but to the presence of a true soluble toxin, which, however, is very resistant to heat.

[1] Ledderhose: Deut. Ztschr. Chirurg., 1888, 28; p. 201.
[2] See Jordan: Jour. Exper. Med., 1899, 4, p. 627.
[3] Breymann: Centralbl. f. Bakt., I, Orig., 1902, 31, p. 481.
[4] Emmerich and Löw: Ztschr. f. Hyg., 1899, 31, p. 1; 1901, 36, p. 9.
[5] Jordan: Jour. Med. Res., 1903, 10, p. 31.

Broth cultures grown at 37° C. assume after a few days a slimy, viscid character, due to the production of "pseudo-mucin."[1] Later the cultures become less slimy and develop true mucin.

Pathogenicity.—For some time after its discovery B. pyocyaneus was generally regarded as a harmless saprophyte, or at worst an organism of slight pathogenic power. Evidence, gradually accumulated, proves this view incorrect, and indicates that B. pyocyaneus is causally associated with a great variety of suppurative and other affections in man. Apart from the many doubtful cases in which B. pyocyaneus is found mixed with streptococci, staphylococci, and other organisms so that its share in inciting pathologic processes is therefore problematic, numerous instances are on record in which little or no question exists as to its etiologic rôle. It has been found by a number of observers in pure culture in abscesses in different parts of the body, especially in the middle ear. Cases of endocarditis and pneumonia have also been met in which B. pyocyaneus seemed to be the sole responsible organism. A generalized and fatal form of pyocyaneus infection has been observed by a number of investigators,[2] and the bacillus has been found in the blood during life.[3] Lartigau[4] found B. pyocyaneus constantly present in the intestinal discharges of patients during a dysentery-like epidemic, and the same organism was also present in abundance in drinking-water which seemed, on epidemiologic grounds, to be implicated in the outbreak. On the whole, there is no doubt that under certain conditions B. pyocyaneus is pathogenic, even gravely so, for man.

Intraperitoneal injection of $\frac{1}{10}$ of a loop of fresh agar culture will kill a guinea-pig with acute symptoms in twenty-four hours. Smaller amounts are also fatal, but less rapidly. Subcutaneous inoculation produces a marked local reaction and is less deadly than intraperitoneal. The symptom-complex presents nothing especially characteristic. Rabbits are not so susceptible as guinea-pigs; mice and pigeons are less susceptible than rabbits. Immunity may be produced by small, non-fatal doses.

[1] Rettger: Jour. Med. Res., 1903, 5, p. 101.
[2] See, for example, Rolly: Münch. med. Wchnschr., 1906, 53, p. 1399.
[3] Brill and Libman: Amer. Jour. Med. Sci., 1899, 118, p. 153.
[4] Lartigau. Jour. Exper. Med., 1898, 3, p. 595.

Proteus

These organisms were originally described by Hauser[1] as an independent genus containing three species—Pr. vulgaris, Pr. mirabilis, and Pr. zenkeri. It is now generally agreed that the last named is not closely related to the typical members of the group and the classification of the Society of American Bacteriologists puts it in a separate genus (Zopfius). At the present time also the first two species of Hauser (separated by him chiefly for differences in the rapidity of gelatin liquefaction) are not regarded as distinct.[2]

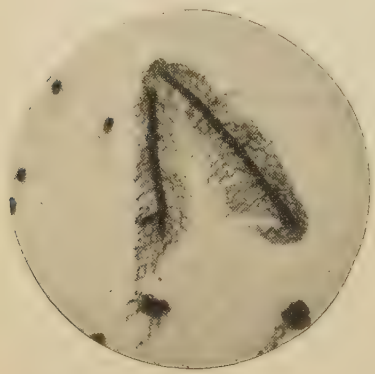


Fig. 109.—Proteus vulgaris, showing flagella (Migula).

Proteus (vulgaris) is one of the most common bacteria in soil and water containing decomposing organic matter of animal origin. It seems to be involved in various commercial processes (see pp. 673, 675). It is often found abundantly in sewage, but does not occur in normal feces so frequently as sometimes stated. It is perhaps to be identified with "the bacterium of putrefaction," the so-called "B. termo" of early writers.

Members of the genus Proteus are gram-negative, usually rodshaped, varying from coccoid to filamentous forms, without endo-spores, actively motile, with peritrichic flagella (Fig. 109). Gelatin is liquefied more or less rapidly, often with a characteristic colony formation with radiating filaments which wander far off into the surrounding medium (Fig. 110). The typical Proteus colonies are best formed when the gelatin is soft, as happens when it is kept at a temperature not far below the melting-point or is made up with 5 instead of 10 per cent. gelatin. Dextrose is fermented with acid

Fig. 110.—Proteus colony (Lehmann and Neumann).

[1] Hauser: "Ueber Fäulnissbakterien," Leipzig, 1885.

[2] Comprehensive studies of the Proteus group have been made by Miss Bengtson: Jour. Infect. Dis., 1919, 24, p. 428; and by Werner and Rettger: Jour. Bacteriol., 1919, 4, p. 331.

and gas production; saccharose and maltose are fermented by some, but not by all, strains; lactose, raffinose, and mannitol are never fermented by the typical Pr. vulgaris. The Voges-Proskauer reaction is negative. Nitrates are reduced. Milk is at first rendered slightly acid, then curdled with alkaline reaction (in about three days), and more or less slowly peptonized.

Proteus was long regarded as especially concerned in the putrefaction of protein substances, but Rettger and Newell[1] have shown that no decomposition of protein is effected by Proteus under anaërobic conditions. With aërobic growth the ordinary non-putrefactive products of protein decomposition are formed.

Proteus has been found connected with a variety of pathologic conditions both in mixed and pure cultures. Infections of the eye and ear, pleuritis and peritonitis, suppurative abscesses in many parts of the body are among the many instances in which the pathogenic power of this organism in pure culture can hardly be doubted. As a producer of cystitis it probably ranks next to B. coli. Besides its independent pathogenicity it is found so commonly associated with other organisms in purulent war wounds and similar processes that its activity as an accomplice is probably second only to that of the cocci.

In certain affections of the digestive tract Proteus has been frequently held to be the responsible agent. In diarrheic stools, especially those of infants, it has often been found in large numbers. Metchnikoff regarded it as the usual cause of infant diarrhea. The real relation of Proteus to intestinal infections is, however, still quite obscure.

A number of food poisoning epidemics have been ascribed to Proteus. These cases have been collected and subjected to a critical analysis by Miss Bengtson,[2] who concludes that a definite proof of the causal relationship between the organism isolated and the illness is lacking in all instances. Further evidence is needed to establish the rôle of Proteus in cases of food poisoning.

Animal inoculation shows that a great range of virulence exists among the Proteus cultures that have been tested. Freshly isolated strains from pathologic sources may produce definite lesions, including abscesses, enlargement of spleen, and a diarrheic condition.

[1] Rettger and Newell: Jour. Biol. Chem., 1912, 13, p. 341.
[2] Bengtson: Jour. Infect. Dis., 1919, 24, p. 428.

One strain from a case of peritonitis has been reported which killed mice in amounts of 0.005 c.c. of broth culture. A very weak, soluble toxin is produced by some cultures.

The use of a strain of Proteus in the diagnosis of typhus (Weil-Felix reaction) is described elsewhere (p. 625).

The Morax-Axenfeld Diplobacillus

A small bacillus (B. lacunatus), about 2 by 1 μ, first described by Morax[1] (1896), and independently by Axenfeld, is responsible for infections of the conjunctiva and cornea in man. The particular

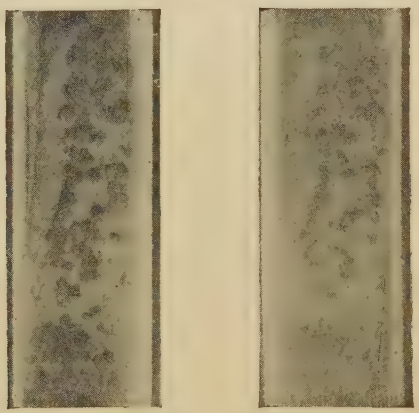


Fig. 111.—Stereoscopic photograph of a culture of the Morax-Axenfeld diplobacillus on blood-serum (Brown Pusey).

eye trouble with which this bacillus is associated is widely distributed, and has been reported in Europe, Africa, and North America. The organism grows readily on Löffler's blood-serum, where it produces in sixteen to twenty-four hours a rather characteristic picture (Fig. 111). The serum is liquefied in a series of pits which are at first separate, but later run into one another. On agar, gelatin, and the other ordinary laboratory media no growth takes place. Stained preparations from cultures show considerable variation in the size of the bacilli; pairs are frequently observed and occasionally short chains (Fig. 112). Involution forms are common after forty-eight hours. The bacilli lose the stain by Gram's method.

So far as known this organism is pathogenic only for the human eye. A blepharo-conjunctivitis, either chronic or acute, is the

[1] Morax: Ann. de l'Inst. Past., 1896, 10, p. 337.

most common condition, but severe inflammation of the cornea is also produced.[1] Treatment with a 0.25 per cent. zinc sulfate

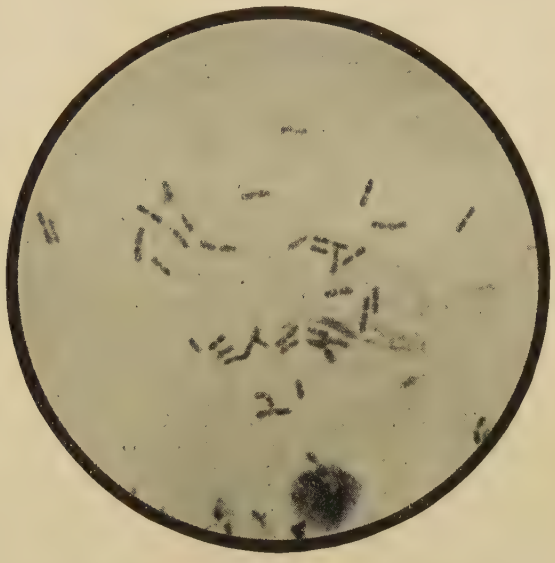


Fig. 112.—Morax-Axenfeld diplobacillus. Smear taken from conjunctiva (Brown Pusey).

solution is specific and produces a rapid cure, while silver salts are without effect (Pusey).

[1] Pusey: Jour. Amer. Med. Assoc., 1906, 47, p. 255.

CHAPTER XXVI

THE PATHOGENIC SPIRILLA

Spirillum Choleræ (Vibrio Choleræ)

Although Asiatic cholera has doubtless smoldered endemically in parts of India for many centuries, the year 1817 marks its first considerable extension beyond the borders of that country. Europe was first invaded in 1831, since which date several great epidemics have carried the disease over a large part of the civilized world. The specific germ was discovered in 1883 by Koch[1] in the intestinal contents of cholera patients.

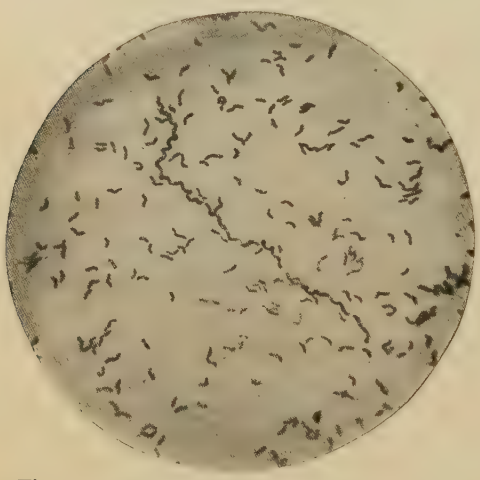


Fig. 113.—Spirilla of Asiatic cholera. Broth culture two days old. Fuchsin stain. × 1000 (Fränkel and Pfeiffer).

Morphology.—In stained preparations the cholera spirillum appears as a short, slightly curved and twisted rod, the so-called "comma bacillus" (Fig. 113). In most cultures some short spirals or S-shaped forms may also be observed. The long, straight, and spiral threads formed in the pellicle of liquid gelatin cultures are usually regarded as involution forms. Cultures that are long transferred on agar often lose the curved form and appear as straight rods, but resume the more characteristic shape when passed through animals. Only a single flagellum is present at one end of the true S. choleræ. Some writers have described cholera germs as possessed of a tuft of several flagella,

[1] Koch: Berl. klin. Wchnschr., 1884, 21, p. 477; Brit. Med. Jour., 1884, 2, pp. 403, 453.

but the organisms observed to be so endowed were not the true cholera spirilla, but some of the numerous related varieties (Kolle). Active motility is manifested. True spores are not found. The cholera spirillum stains readily with the ordinary anilin dyes. It is decolorized by Gram's method.

Cultural Characters.—Growth occurs abundantly on the ordinary culture-media, a distinctly alkaline reaction, however, being essential. Upon gelatin plates at 20° to 22° C. the growth is quite characteristic. The colonies are plainly visible to the naked eye after twenty-four hours, and are at first round, with an even contour, later becoming irregular; under a low power of the microscope the colony presents a rough, granular appearance, as if the surface were covered with fine particles of glass (Fig. 114). A little later—within forty-eight hours—liquefaction of the gelatin sets in, and the colony sinks into a funnel-shaped depression whose area rapidly extends. This typical colony formation occurs in most of the freshly isolated cultures, but considerable variation sometimes takes place; in old laboratory cultures liquefaction is tardy and the colonies have a brownish tinge.

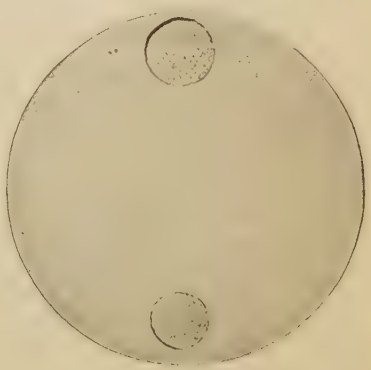


Fig. 114.—Colonies of the cholera spirillum. Gelatin, twenty-two hours old; × 100. Zettnow prep. (Kolle and Wassermann).

Stab-cultures in gelatin often develop a small, turnip-shaped area of liquefaction at the surface, which by evaporation of the fluid leaves a bubble-like depression, while in some growths but little or no liquefaction occurs along the needle puncture. Other vibrios besides the cholera germ, however, produce this same type of liquefaction; and the cholera spirillum itself sometimes liquefies tube cultures in a different manner.

On agar plates the colonies may be readily distinguished from those of B. coli by their thin, opalescent appearance. This feature is of importance in effecting a speedy diagnosis by means of plates made directly from feces.

Blood-serum is rapidly liquefied. On potato at 37° C. a viscid brownish growth is produced. In broth growth is luxuriant; the

medium becomes rapidly clouded, and a wrinkled pellicle is formed at the top. In milk growth takes place without producing any visible change. When the cholera spirillum is grown in peptone broth containing a small amount of nitrate,[1] the nitrate is reduced to nitrite, and at the same time indol is produced. The addition of a few drops of concentrated sulfuric acid to the culture then gives rise to a red color—the cholera-red reaction. This was at one time supposed to be distinctive of the cholera spirillum, but is now known to be given by a number of other vibrios, so that as a positive test it is not diagnostic. Its absence, however, is possibly of some importance in the identification of the cholera germ, provided nitrite is known to be present at the time the test is made. The reaction itself is none other than the familiar nitroso-indol reaction, and is given by any organism (*e. g.*, the colon bacillus) that forms indol and reduces nitrate.

The cholera spirillum is a strongly aërobic organism and refuses to grow under a strip of mica laid over a gelatin plate; in fluid cultures the vibrios collect at the surface. An alkaline reaction of the culture-medium is also essential to its development, a small quantity of acid being sufficient to prevent growth altogether.

The resistance of the cholera vibrio to various injurious influences is not great. It is killed by moderately high temperatures very readily (ten minutes at 60° C.), is destroyed quickly by the chemical disinfectants, and does not retain its vitality long in association with the ordinary saprophytic bacteria of the soil or water. Toward desiccation it is especially sensitive; if a drop of broth culture be dried on a cover-slip, the vibrios are all dead in about two hours. The facts which attest the slight resistance of the cholera spirillum, especially its sensitiveness to drying, explain the rapid and complete disappearance of cholera in once infected localities, and also the circumstance that the disease is rarely, if ever, transmitted by aërial infection. Whether or not the cholera vibrio is able to multiply outside the body in impure water is in doubt. In some peaty river-waters at least, as shown by Hankin for the Ganges and Jumna rivers, the conditions for multiplication, and even for the continued vitality of the organism, are highly unfavorable. Upon the surface of vegetables and fruits kept in a cool

[1] This may be present as an impurity in the table-salt or some other ingredient used in making the nutrient broth, or may be specially added.

moist place experiments have shown that the spirillum may retain its vitality for from four to seven days.

The Examination of Feces for the Cholera Vibrio.—Rapid, practical methods for the detection of cholera vibrios in stools have been developed in this country by McLaughlin, Stimson, and others, in connection with the examination of large numbers of steerage passengers at quarantine. The specimen may be secured by the use of an aperient (magnesium sulfate on an empty stomach at 6 A. M.), or by use of a long rectal tube with several eyes cut at the upper end. Some workers consider that the dose of salts may be dangerous if given to a carrier with vibrios in the intestine by reason of the possible lowering of resistance it may bring about, but McLaughlin[2] states that he administered salts in about 2000 cases at Boston and Providence without observing any ill effects.

A quantity of feces estimated to weigh about 1 gram (1 c.c.) is then added to 100 c.c. of sterile peptone solution[1] and incubated six hours at 35° to 37° C. Smears from the surface film should then be made (not later than eight hours), stained with carbol-fuchsin, and examined. In the hands of a skilled observer[2] the method leads to the elimination of 80 to 90 per cent. of the specimens without plating. "The observer must search, using a mechanical stage, from twenty-five to fifty fields, and if he finds no suspicious curved organism, the specimen is a marked negative. If he finds curved organisms, subcultures in peptone and plates are made" (McLaughlin). It is well to make platings from the surface of the peptone solution six, twelve, and eighteen hours after inoculation. Colon bacilli grow feebly, if at all, on this medium. Cholera vibrios grow luxuriantly, but so do other related vibrios.

Goldberger[3] found in a series of comparative tests that the cholera organism could be recovered from feces in the presence of very large numbers of the ordinary fecal bacteria by use of an alkaline-egg-peptone solution. This is made up as follows:

"(*a*) Prepare an alkaline-egg solution by first shaking up or beating up an egg with an equal volume of water and then adding

[1] McLaughlin gives the following formula: Peptone (Chapoteau or Witte), 10.0; sodium chlorid, 10.0; potassium nitrate, 0.1; sodium carbonate, 0.2; distilled water, 1000. Reprint Public Health Rep., No. 53, Washington, 1910.

[2] McLaughlin: Bost. Med. and Surg. Jour., 1911, 165, p. 561.

[3] Goldberger: Bull. No. 91, Hyg. Lab., Washington, 1913, p. 19.

to this egg-water an equal volume of a 5 per cent. solution of *anhydrous* sodium carbonate. Steam for three-quarters to one hour. (*b*) Prepare Dunham's solution: Peptone 10, salt 5, water 1000.

"For use mix (*a*) and (*b*) in proportion of 1 : 9. Run through paper filter; distribute in 10 c.c. quantities in tubes and sterilize by steaming for one and one-half hours, after which they are ready for use. This solution is of a pale straw color. It is opaque, and a slight precipitate settles to the bottom of the tube on standing, but this interferes in no way with its serviceability. It will keep at least a week."

For plating, the elective medium of Dieudonné[1] is often used, but ordinary nutrient agar is about as satisfactory. The plates of agar should be poured and then dried for an hour in the incubator before inoculation. The peptone culture is best smeared over the surface with a bent glass rod and the plates incubated at 36° to 37° C. for sixteen hours. Control platings with a known cholera vibrio should always be made until the worker is assured of his ability to recognize the colonies.

Final identification depends on the agglutination test, which should preferably be carried out with a serum of rather high titer (at least 1 : 4000). The usual precautions necessary for this test should always be observed (p. 180), and control tests with a known vibrio and with normal serum should be made.

By such a method it is possible to make an examination of every passenger in a short time. In August, 1911, the steamer Canopic arrived at Boston from Naples and a bacterial examination of 1193 second-class and steerage passengers was concluded in fifty-four hours.

Cholera Carriers.—In regions where cholera exists healthy persons sometimes have cholera vibrios in the intestines. As a rule, the vibrios are not found in the stools longer than ten days, and very rarely over twenty days, after convalescence; but one case is on record[2] where vibrios were found for sixty-nine days, and several other observers have reported a duration of about fifty days. Such facts prove the possibility of the conveyance of cholera infection over long ocean voyages, even though no definite case of

[1] Dieudonné: Centralbl. f. Bakt., I, Orig., 1909, 50, p. 107.
[2] Bürgers. Hyg. Rundsch., 1910, 20, p. 169.

cholera develops on shipboard. In point of fact, as many as 4 cases developed in the United States in the summer of 1911 among persons who had passed quarantine inspection. Two of these cases had been detained in quarantine at the port of New York for as long as seven days. One case developed on Staten Island, N. Y., in an employee who had previously been guarding apparently well persons at quarantine.

Cholera carriers are naturally more abundant in times and places where cholera is prevalent than when only scattered cases exist. McLaughlin[1] found that 17 out of 264 healthy persons at Bilibid Prison, Manila, were carrying cholera vibrios at a time when the disease was epidemic in the prison. The outbreak was quickly suppressed by enforcing the thorough disinfection of the hands upon leaving the latrines and before eating.

Intermittent discharge of cholera vibrios is sometimes observed. In a case reported by Creel[2] the cholera vibrio was present in four and absent in six examinations made during a period of four weeks following the first negative examination after recovery. Greig[3] has found the cholera vibrio in the bile in no less than 80 out of 271 fatal cases, and believes that chronic cholera carriers, like typhoid carriers, owe their prolonged infectivity to the persistence of the bacteria in the gall-bladder.

Pathogenicity for Man.—The causal connection between Asiatic cholera and the micro-organism discovered by Koch has been demonstrated by a number of unfortunate laboratory accidents affecting persons working with pure cultures of the microbe in question. One of these cases occurred in Hamburg in September, 1894, at a time when there were no other cases of the disease in that city.[4] Dr. Emil Oergel, assistant in the Hamburg Hygienic Institute, became accidently infected in the course of his laboratory work, probably by drawing into his mouth through a pipet some of the peritoneal fluid from an inoculated guinea-pig with which he was experimenting. Clinically, the patient presented a typical picture of severe cholera; a nearly pure culture of the cholera vibrio was found in the stools, and about nine days after the probable infection the case terminated fatally. In another German laboratory two

[1] McLaughlin: N. Y. Med. Jour., 1911, 93, p. 115.
[2] Creel: Jour. Amer. Med. Assoc., 1912, 58, p. 187.
[3] Greig: Indian Jour. Med. Res., 1913, 1, p. 44.
[4] Reincke: Deut. med. Wchnschr., 1894, 20, p. 795.

experimenters, Pettenkofer and Emmerich, voluntarily partook of a small quantity of a broth culture of "Koch's vibrio," and as a result developed mild but genuine cases of Asiatic cholera. By such instances the relation between Asiatic cholera and the particular spirillum has been demonstrated with sufficient emphasis.

Both laboratory cases of cholera and those cases contracted naturally in the course of epidemics are marked by great differences in the susceptibility of different individuals. This probably is due partly to individual differences in natural resistance, partly to the fluctuations that occur in such resistance. It is well known that when cholera is prevalent, certain persons contract the disease and succumb to it more readily than others who seem equally exposed to infection. Fatigue, the excessive use of alcohol, and various factors leading to mild, non-specific gastro-intestinal derangements, predispose in a marked degree to attacks of cholera.

The tissue changes observed at autopsy are not characteristic. Kidneys and liver frequently show cloudy swelling, but this is not invariable. The intestinal tract, especially the lower half, is sometimes congested, sometimes affected with extensive necrosis and formation of a false membrane; the latter condition is seen particularly in the more chronic cases.

The patient's serum cannot be satisfactorily used for a diagnostic agglutination test as in typhoid fever,[1] because the agglutination property of the serum is irregular in appearing and may not develop at all. A fall of the opsonic index occurs with the onset of cholera, as of other diseases.

The cholera spirilla usually occur in immense numbers in the contents of the intestines, but do not invade the blood, and are not found, as a rule, in the internal organs. They penetrate to some extent, however, into the wall of the intestine itself, and loosen by their action the epithelial cells, which are shed into the intestine, giving the characteristic "rice-water" appearance to the stools and intestinal contents. In the rice-water stools the spirilla are often found in pure culture, but in later stages, and in the so-called cholera-typhoid condition, other bacteria become so abundant that the isolation of the cholera spirillum from choleraic dejecta becomes much more difficult. Greig[2] has reported the occurrence of the cholera vibrio in the urine of cholera patients.

[1] Svenson: Ztschr. f. Hyg., 1909, 64, p. 342.

[2] Greig: Indian Jour. Med. Res., 1913, 1, p. 90.

Fig. 115.—Chart of the Hamburg

lera epidemic (Kolle and Hetsch).

Modes of Dissemination; Epidemiology.—Cholera, like typhoid fever, is spread largely by means of infected drinking-water. The short period of incubation and the rather unmistakable clinical picture often render the water-borne epidemics of cholera more easily traceable to their source than those of typhoid fever. The explosive character of such outbreaks is usually striking. In the famous "Broad Street pump" epidemic of cholera in London in 1854,[1] there were in the district affected twelve deaths from cholera during a period of thirteen days preceding the outbreak; then in four successive days the number of deaths leaped to three hundred and forty-four. A similar explosive outbreak occurred on August 20, 1892, in Hamburg, when the water-supply of that city became infected (Fig. 115). The epidemic at Hamburg affords particularly strong evidence of the relation between drinking-water and cholera, as shown by the following facts. The neighboring city of Altona, which is separated from Hamburg only by a political—not by a social or topographic—boundary-line, derives its water-supply from the river Elbe, purifying it by sand-filters. The Hamburg supply in 1892 came from the same stream, but at that time was not filtered. Throughout the period when Hamburg suffered severely from cholera Altona remained practically exempt. Houses on one side of a street which were supplied with water from the Hamburg mains developed many cases of cholera, while those on the other side, although under identical social and climatic conditions, built on the same soil, and provided with the same sewerage system, *but supplied with Altona water*, remained free.[2]

Milk and other articles of food ordinarily consumed in an uncooked state may likewise be the vehicle by which the cholera germ is conveyed into the alimentary tract. Cases of cholera due to contact infection sometimes occur, those persons in the immediate household of cholera patients, and especially those concerned in the care of the latter, being always more or less liable to become infected.

The explanation of the epidemiologic relations of cholera is found in the bacteriologic observations: (1) That cholera spirilla leave the body of the patient in the feces (rarely in the urine); (2) that not only patients and convalescents from cholera, but also healthy

[1] Report on Cholera Outbreak in the Parish of St. James, Westminster, during the autumn of 1854. London, J. Churchill, 1855.

[2] Koch: Ztschr. f. Hyg., 1893, 14, p. 393.

individuals from cholera-infected regions, may pass cholera spirilla in their bowel discharges. (See p. 484.) These facts make it clear why sewage-polluted water is often the cause of outbreaks of cholera, why the soiling of hands, garments, bed-clothing, etc., can lead to contact infection, and why cholera can suddenly arise at a point remote from other centers of infection as a consequence of the arrival of travelers from a cholera-ridden district. There is no mysterious influence of "locality" in cholera epidemics except that the germ is introduced into certain places and not into others, and that, when once introduced, its wide dissemination is favored by certain factors, such as a polluted public water-supply, defective disposal of excremental refuse, or the unsanitary conditions that are fostered by overcrowded dwellings and extreme poverty.

So far as known, infection occurs only through the alimentary tract; the germ must be swallowed. The danger of dissemination by dust is slight, owing to the feeble resistance shown by the germ to drying. On the other hand, contamination of exposed food through the agency of flies is a well-grounded probability.

Animal Inoculation.—Under natural conditions domestic animals and the animals used in laboratory experimentation never contract cholera. Somewhat drastic methods, such as alkalinization of the stomach contents with soda and slackening of the peristaltic movement of the intestine with opium, were employed by the earlier experimenters in their attempts to produce infection, and in some cases (guinea-pigs, Nicati and Rietsch[1]) these apparently succeeded. It was subsequently shown, however, that the choleraic symptoms and fatal termination produced in this way could also be brought about by other vibrios, and were not a peculiar manifestation of the true cholera spirillum.

Experiments with rabbits have given the most important results. Injection of a very small quantity of a culture of cholera vibrios into the ear-vein results in the death of these animals with intestinal lesions not unlike those observed in man. Young rabbits may be infected through the mouth in a large proportion of cases by the simple expedient of placing cholera spirilla on the teats of the mother (Metchnikoff). Animals so infected present the characteristic features of typical cholera.

[1] Nicati and Rietsch: Deut. med. Wchnschr., 1884, 10, p. 634.

Intraperitoneal injection of guinea-pigs, while it does not reproduce typical Asiatic cholera, gives rise to a fatal infection which has been exhaustively studied by Pfeiffer[1] and other workers. The virulence of cholera cultures is frequently tested in this way, and a number of important discoveries relating to the mechanism of immunization and the presence of bactericidal substances in the serum have been made in connection with the study of "intraperitoneal cholera."

Toxin.—For a long time no poisonous substance akin to those contained in the broth cultures of the diphtheria and tetanus bacilli was detected in cultures of the cholera spirillum. Pfeiffer, who was one of the first workers in this field, found that young cultures in fluid media were practically devoid of toxicity. On the other hand, young agar cultures freshly killed with chloroform vapor or by heating to 56° C. were found to contain labile toxic substances. On such grounds it was inferred that the cholera spirillum produces no true toxin, but contains an endotoxin or poisonous body closely bound to the cell substance.

Metchnikoff, Roux, and Taurelli-Salimbeni,[2] however, found that if cholera spirilla were placed in collodion sacs in the peritoneal cavity of guinea-pigs, multiplication of the bacteria occurred within the sac and the animals died without the advent of any spirillæ into the organs, blood, or peritoneal exudate. This they attribute to the production by the living cholera spirilla of a soluble toxin which diffuses through the wall of the collodion sac. Brau and Denier[3] and Kraus and Russ[4] have obtained yet more decisive results, and have demonstrated the presence of a true toxin in broth cultures. The serum of actively immunized animals neutralizes the cholera toxin, and hence presumably contains a true antitoxin. In animal experiments the cholera antitoxin exerts a curative effect.

Vaccination Against Cholera.—It is possible to immunize guinea-pigs and other animals by incorporating in their bodies, subcutaneously or intraperitoneally, living cholera bacteria, either of full virulence or attenuated. Bacteria killed by a moderately high

[1] Pfeiffer: Ztschr. f. Hyg., 1894, 18, p. 1; 1895, 20, p. 198.

[2] Metchnikoff, Roux, and Taurelli-Salimbeni: Ann. de l'Inst. Past., 1893, 7, pp. 403, 562; 1894, 8, pp. 257, 529.

[3] Brau and Denier: Ann. de l'Inst. Past., 1906, 20, p. 578.

[4] Kraus and Russ: Centralbl. f. Bakt., Abt. I, Orig., 1907, 45, p. 258.

temperature or by chloroform may also be used. Filtered germ-free cultures of S. choleræ, unlike those of B. diphtheriæ, possess no immunizing power. From this it is inferred that the immunity-stimulating substance, which is usually identified with the specific toxin, is closely bound to the cell body. Whatever method is employed, whether injection of living spirilla or injection of those killed by chloroform or heat (fifteen minutes at 65° C.), it is important to graduate the dose, increasing by small amounts the quantity injected. A method commonly employed in animal experimentation is to make first several injections of killed spirilla, and then, when a certain degree of immunity has been obtained, to inject living virulent spirilla at suitable intervals (*e. g.*, seven days) in gradually increasing doses.

The immunity so conferred is not accompanied by the development of any antitoxic quality in the blood of the immunized animal. This is shown by the fact, among others, that an animal immunized to a high degree against living cultures succumbs just about as readily as a normal animal to inoculation with dead cultures. The active power of the immune serum depends upon its ability, when injected along with cholera spirilla into the peritoneal cavity of a guinea-pig, to cause the death and disintegration of the spirilla. Cholera immune serum is bacteriolytic, not antitoxic. The serum of an immunized animal (goat, Pfeiffer) may be so potent that 0.0001 c.c. is sufficient to protect a guinea-pig against intraperitoneal injection with ten times the minimum fatal dose of cholera spirilla. The bactericidal property of the immune serum is specific: anti-cholera serum will not destroy S. metchnikovii or other vibrios related to, but not identical with, the true cholera spirillum.

While the serum produced in this way possesses considerable preventive, it has little or no curative, power. If within one-half hour after intraperitoneal injection with a loop of a virulent cholera culture a guinea-pig is injected with anti-cholera serum of high potency, the life of the animal is saved; if, however, a period of one and one-half hours be allowed to elapse, large doses of serum are of no avail. In the latter case, indeed, the spirilla are destroyed, but the time interval has given such an opportunity for multiplication that the amount of poisonous substance liberated from the bodies of the dissolved bacteria is enough to cause death. After

a still further interval the bacteriolytic action is much lessened, and finally is no longer manifest.

The results obtained in man with the serum of immunized animals are in general accord with the outcome of animal experimentation as above related. The principle of passive immunization, which is so effective in the case of diphtheria, has not as yet come into general use in cholera, although the work of Kraus and Russ indicates the applicability of the method, and the possibility of its future extension.

Active immunization of human beings has, on the contrary, been advocated and rather extensively practised. Passing over the work of Ferran (1884) as of little practical importance on account of his use of impure cultures and other loose methods of experimentation, the first really noteworthy venture in this direction is the work of Haffkine.[1] This experimenter used first a weak virus, that is to say, a culture attenuated by long cultivation or by growth at a high temperature (39° C.), and then followed this five days later with a virulent culture, both inoculations being made subcutaneously. Haffkine's main field of application of his method has been that home of cholera, British India, where many thousands of Europeans and natives have been vaccinated. The success of this treatment as a prophylactic method is attested by a large body of statistics which, while leaving something to be desired on the score of comparability and accuracy, nevertheless carry conviction on the main point. The following illustration of the incidence of cholera upon the two groups of vaccinated and unvaccinated in a certain part of India will show the nature of the evidence (Powell[2]).

	Number	Cases	Deaths
Unvaccinated	6549	198	124
Vaccinated	5778	27	14

A similar method of vaccination was devised by Kolle,[3] which consists in inoculation with cultures killed by heating to 58° C. for an hour. The efficacy of this method has been tested to some extent by examination of the bacteriolytic power of the serum of vaccinated persons. Kolle's method was extensively used in an epidemic of

[1] Haffkine: Brit. Med. Jour., 1895, 2, p. 1541.
[2] Powell: Jour. Trop. Med., 1899, 2.
[3] Kolle: Deut. med. Wchnschr., 1897, 23, p. 4.

cholera in Japan, where the results were, if anything, somewhat more favorable than the results obtained in India.[1]

The mode of action of cholera-immune serum upon the cholera vibrios has been thoroughly studied by Pfeiffer,[2] Ehrlich, and others. When brought in contact with the serum, the bacteria successively lose their motility, become swollen and paler, and break up into rounded, coccus-like granules which finally dissolve and disappear altogether. These changes are observed not only in spirilla introduced into the peritoneum of an immunized guinea-pig, but also in spirilla mixed with fresh immune serum outside of the body. The test-tube experiments have afforded a means of studying the mechanism of bacteriolysis. Two substances were shown by Ehrlich to be concerned in the lytic process. One of these, the complement, is a labile substance contained in abundance in the serum of normal animals; the other, the amboceptor, a more stable body, is developed in the organism during the process of immunization, although there is evidence that small amounts of amboceptor occur in the normal animal. Neither substance alone can destroy the cholera vibrio. The relation of these two substances is shown by the following table, further details concerning the action of the cholera and other bactericidal sera being given in the chapter on Immunity (pp. 159–164):

Normal serum (Complement + small amount of Amboceptor)	Slight bacteriolysis.
Immune serum (Complement + Amboceptor)	Bacteriolysis.
Heated immune serum (Amboceptor only, Complement destroyed)	No bacteriolysis.
Heated immune serum plus normal serum (Amboceptor + Complement)	Bacteriolysis.

Allied Varieties.—A number of vibrios more or less closely resembling the cholera spirillum have been found in the stools of persons suffering from cholera, in sewage, and in polluted water. Some of these exhibit slight differences in size, number of flagella, ability to reduce nitrate, to produce phosphorescence, etc., and have been given names such as S. Ghinda, S. Massauah, S. Danubicus, S. phosphorescens, etc., which refer to some biologic peculiarity, or to the locality where the vibrios were found. The agglutinative

[1] Murata: Centralbl. f. Bakt., I, Orig., 1903–04, 35, p. 605.

[2] Pfeiffer: Ztschr. f. Hyg., 1894, 18, p. 1; 1895, 20, p. 198; Sobernheim: Ibid., p. 438.

and bacteriolytic tests have afforded a means for distinguishing between these allied varieties and the true cholera spirillum, and less emphasis is now laid upon other points of divergence and resemblance than was the case at an earlier period. The organism known as S. Massauah, cultivated by Pasquale from human dejecta (non-choleraic), and S. Ghinda, found in well-water, were for a long time considered as true cholera germs, but have been shown to respond negatively to the serum test. It must still be regarded as an open question how far these cholera-like vibrios found in persons suffering from cholera or a cholera-like disease are responsible for the symptoms with which they are associated. From analogy with the typhoid group of diseases, as well as for other reasons, it seems probable that very similar, if not practically identical, symptoms and lesions may be produced by micro-organisms differing in agglutinative and bacteriolytic affinities. The majority of the cholera-like vibrios, so far as studied, however, are probably essentially saprophytic forms, or, at most, possessed of slight pathogenic power.

An apparent exception to the last statement was for a time thought to exist in the case of the famous El Tor vibrios[1] isolated from the bodies of some pilgrims who died with dysenteric symptoms at a time when no cases of cholera were known in the vicinity. These vibrios resemble true cholera vibrios in many respects, but differ in having a marked hemolytic action and in producing a powerful extracellular toxin. They respond positively to the agglutinative and bacteriolytic tests, and to the complement fixation reaction. There is now general agreement that the El Tor vibrios are true cholera vibrios, differing only quantitatively from the types earlier studied.

Spirillum Metchnikovii

The organism known by this name was first discovered by Gamaléia[2] in the intestinal tract and blood of fowls suffering from an epidemic disease resembling fowl cholera. It is extraordinarily like the cholera spirillum in all its morphologic and cultural characters, practically the only difference observed being in the colonies on gelatin plates, which, in the case of S. metchnikovii, sometimes have a brownish tinge, show turbidity of the liquefied gelatin, or diverge in other slight particulars.

[1] F. Gotschlich: Ztschr. f. Hyg., 1906, 53, p. 281.

[2] Gamaléia: Ann. de l'Inst. Past., 1888, 2, p. 482.

Inoculation of pigeons affords a ready means of distinguishing this organism from S. choleræ. Pigeons are unaffected by injection of relatively large numbers of the latter bacterium, but usually succumb within twenty-four hours to septicemia when pricked with a needle dipped in a culture of S. metchnikovii (Fig. 116). For guinea-pigs also the pathogenicity of S. metchnikovii is higher than that of the cholera vibrio. These animals, on subcutaneous inoculation, fall victims to a rapidly fatal septicemia; if the stomach contents are rendered alkaline, infection can also be produced by feeding.

Further evidence of the difference between these forms is given by the agglutinative reaction and Pfeiffer's serum test. The serum of an animal immunized against intraperitoneal cholera will not protect against S. metchnikovii, and will not agglutinate it. The reciprocal statement is also true. Vibrios similar to, and perhaps fully identical with, S. metchnikovii have been isolated by several observers from polluted water. "Vibrio Massauah," an organism long maintained in laboratory collections under the impression that it was a strain of the true cholera spirillum, is now regarded as more closely related to S. metchnikovii.

Fig. 116.—Spirillum metchnikovii in exudate from pigeon. × 1000 (Günther).

The "spirillum of Finkler and Prior" was originally discovered in old stools obtained from a case of cholera nostras.[1] It does not give the typical cholera-red reaction, and may be differentiated in other ways from the cholera spirillum. At present the microbe has only a rather remote historical interest, and would hardly need to be mentioned save for the fact that it has long been cultivated in bacteriologic laboratories, and consequently used to some extent in comparative and experimental work. The same may be said of "Deneke's spirillum," the so-called S. tyrogenum. The latter organism has very feeble pathogenic power for laboratory animals;

[1] Deut. med. Wchnschr., 1884, 10, p. 632.

the spirillum of Finkler and Prior usually displays more, but never a high, virulence.

A phosphorescent vibrio (S. phosphorescens) isolated from river-water in parts of Germany possesses a decided resemblance to the cholera spirillum, but besides giving luminous cultures, is not influenced by specific cholera serum.

CHAPTER XXVII

THE SPIRÓCHETES

Spirally shaped micro-organisms were for a long time grouped together indifferently under the common name spirillum, spirochete, or vibrio. Further knowledge, however, has shown that certain of these forms possess characteristics that distinguish them, on the one hand, from undoubted bacteria, such as the cholera vibrio, and on the other from actively motile protozoa, like the trypanosomes. While there are still some observers who regard the "spirochetes" of syphilis and relapsing fever as plant organisms, and others who regard them as animals, opinion has been shifting to the belief that these organisms stand somewhat apart from both Bacteria and Protozoa, and should perhaps be regarded as constituting an independent group between the two. Classification and nomenclature within the group are still in an uncertain condition, but the table of classification (pages 500, 501) based on Noguchi's studies may be regarded as giving a good working basis.

Study of the spirochetes has been much stimulated by the discovery of the germ of syphilis, which belongs to this group, and by the use of the dark-field microscope (p. 601), which has overcome many of the difficulties in the study of the spirochetal organisms, difficulties caused by the extreme tenuity and unsatisfactory staining qualities of these forms.

Micro-organisms apparently belonging with the spirochetes are the large saprophytic commensal forms (Cristaspira), occurring in the alimentary canal of certain mollusks, such as the oyster and the fresh-water mussel. Among the known spirochetes pathogenic for man are the spirochetes of the relapsing fevers, infectious jaundice, and rat-bite disease (Spironema), the spirochetes of syphilis and yaws (Treponema), and Noguchi's spirochete of yellow fever (Leptospira).[1]

[1] A full discussion of classification and nomenclature has been given by Noguchi in his Harvey Lecture (Science, XI, 1915–16, p. 174) and in the Journal of Experimental Medicine, 1918, 27, p. 575.

The Spirochetes of the Relapsing Fevers (Spironema)

In 1873 Obermeier[1] announced the discovery of a large spirillum in the blood of patients suffering from a peculiar disease known as relapsing fever, which has been known since the early part of the eighteenth century, and has at times prevailed extensively in parts of Europe. The characteristic feature of this affection is that after apparent recovery and the cessation of all active symptoms one or more relapses invariably follow. Similar maladies

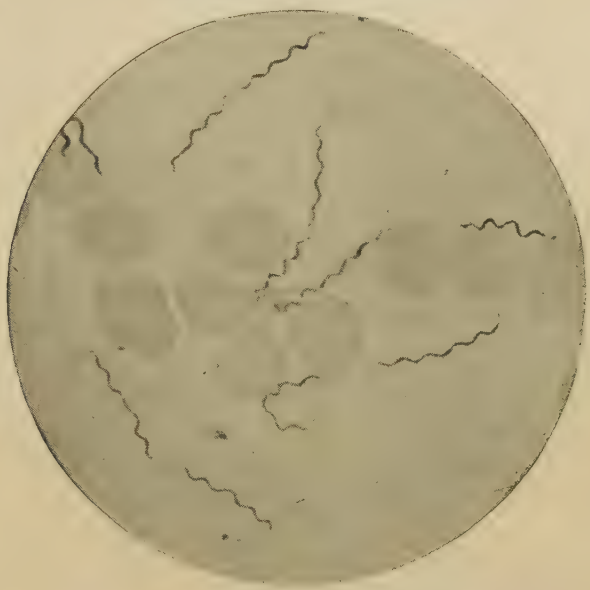


Fig. 117.—Spironema obermeieri in blood of rat, showing divisional forms. A group showing spirals of different lengths. The two long ones above show transverse division; × 1500 (Novy and Knapp).

are common in India, Africa, and other parts of the world at the present time. In recent years few cases have been observed in the United States.[2] The European variety of the disease is not a very fatal one, the case-mortality being stated by Murchison[3] to be about 4 per cent.

S. Obermeieri: Chief Characteristics.—Spironema obermeieri (S. recurrentis) is a tapering, spiral filament, varying in length from

[1] Obermeier: Centralbl. f. d. med. Wissensch., 1873, 11, p. 145.

[2] For a careful report of two cases see Carlisle: Jour. Infect. Dis., 1906, 3, p. 233.

[3] Murchison: "A Treatise on the Continued Fevers of Great Britain," London, 1873.

one and one-half times (probably the young single cell) to ten times the diameter of a red blood-corpuscle (agglutinated cells). The breadth of the cell is about 0.39μ. The number of turns in the short spirals or individual cells is usually only two or three, but may increase to eight to ten prior to division. The individual spirals have a single flagellum at one end, and display a very active screw motion, with some lateral oscillation and, under favorable conditions, rapid forward-backward excursions (Figs. 117 and 118). The contents of the cells usually stain uniformly by the Romanowsky method, with the exception of the ends, which are pale and fade away to a point (Novy). These spirochetes have been cultivated outside the body by Noguchi. His procedure consists in adding a few drops of citrated rat or mouse blood containing the spirochetes to sterile ascitic or hydrocele fluid (10 to 15 c.c.) containing pieces of fresh rabbit kidney. Best results were obtained when the infected blood was drawn between the forty-eighth and seventy-second hour of the attack, and the tube incubated at 36° C. The avoidance of bacterial contamination is essential to success. Several strains of relapsing fever parasites from different parts of the world were cultivated in this way. The parasite of African relapsing fever (S. duttoni) was still virulent after the ninth transfer. Plotz[1] has succeeded in cultivating S. obermeieri directly from human blood.

Fig. 118.—Spironema obermeieri in blood of rat. Shows a long free flagellum at one end and a rudimentary one at the other. Löffler's stain. Note that the flagellum seems to be given off from the side of the tip; × 3000 (Novy and Knapp).

Relationship and Nomenclature.—Schaudinn[2] was first to assert that the special organism seen in relapsing fever belonged not to the bacteria, but to the protozoa, basing this opinion on its alleged possession, like trypanosomes, of a definite nucleus, blepharoplast, an undulating membrane, and flagellum. Novy and

[1] Plotz: Jour. Exper. Med., 1917, 26, p. 37.

[2] Schaudinn: Arb. a. d. k. Gesund., 1904, 20, p. 387.

Knapp[1] and others, however, have shown that this view is incorrect: no nucleus, blepharoplast, or undulating membrane could be demonstrated by these later observers. The flagellum is like that of bacteria in general and is not of the protozoan type; multiplication takes place by transverse, not longitudinal, division, and is very rapid; toward plasmolytic agents and toward heat S. obermeieri behaves like a bacterium, not like one of the protozoa; active immunity of a high degree is readily established. On the other hand, the immunity reactions, the behavior of the parasites toward certain chemicals, and the course of the infectious process seem to indicate an affinity with the protozoa.

Pathogenicity.—The spirochetes of relapsing fever can be transmitted by inoculation to man, monkeys, mice, and rats. Carlisle[2] describes a case in man occurring as the result of accidental inoculation, probably from the bite of a monkey in the course of experimental work with the spirochete. A typical paroxysm, with severe frontal headache, sharp pains in the back, and fever, was followed by a critical defervescence, ending apparently in complete recovery. In about a week a second paroxysm occurred, and then, after a remission, a third. This is fairly typical of the course of cases of relapsing fever observed in an epidemic. Sometimes four or even more relapses occur. The spirochetes are found in the blood during the febrile paroxysm, but tend to diminish in number during succeeding relapses.

Certain monkeys may be infected by subcutaneous inoculation with human or animal blood containing spirochetes. The infection in these animals, like the naturally contracted relapsing fever in man, is characterized by successive attacks of illness, usually three or four in number, accompanied by the appearance of spirochetes in the peripheral blood. The rise in temperature at the time of the paroxysm is less marked and less constant in monkeys than in cases of human relapsing fever. The course of the disease is usually benign, the inoculated animals speedily regaining their health after the last paroxysm.

White mice are very susceptible to inoculation, and typical relapses occur in these animals. White rats also are readily

[1] Novy and Knapp: Jour. Infect. Dis., 1906, 3, p. 291.
[2] Carlisle: Jour. Infect. Dis., 1906, 3, p. 233.

CLASSIFICATION OF SPIRAL ORGANISMS

	Spirochæta ("coil-hair")	Saprospira ("putrid-coil")	Cristaspira ("crested coil")	Spironema ("coil-thread")	Treponema ("to turn, thread")	Leptospira ("fine-coil")
Measurements in microns — Length	100 to 150	100 to 120	45 to 90	8 to 16	6 to 14	7 to 9 or 14
Measurements in microns — Diameter	0.5 to 0.75	.....	1.0 to 1.5	0.35 to 0.5	0.25 to 0.3 Cylindrical	0.25 to 0.3
Measurements in microns — Width of spiral	2	.....	.....	.....	1.0 Regular Rigid	0.45 to 0.5
Measurements in microns — Length of spiral	1.5 Regular	.....	.....	.....	0.8 to 1.0 Very constant	0.3 Regular
Shape of ends	Blunt	.....	Blunt	Pointed	Pointed	Pointed
Waves (in addition to finer spirals).	Several large, inconstant, irregular.	Large, inconstant, shallow, irregular, 3 to 5 in number.	Two to five or more. Large, irregular shallow.	Large, wavy spirals, usually five.	One or more slight undulating waves.	One or more gentle wavy curves throughout entire length. One or both ends semicircularly hooked when in liquid media. Serpentine in semi-solid media. Extremely flexible.
Axial filaments	Present (flexible elastic)	Absent	Absent	Present (?)	Doubtful	Absent
Chambered structure	Absent	Present	Present	Absent	Absent	Absent
Membrane	Absent	Present (flexible elastic)	Present (flexible elastic)	Delicate flexible double contoured	Doubtful	Absent
Terminal (finely spiral filament)	Absent	Absent	Absent	Present	Present	Not recognized

Flagella	Absent	Absent	Absent	Absent	Absent	Absent
End (highly motile end portion).	Absent	Absent	Absent	Absent	Absent	Well developed in last 6–8 spirals.
Crista (undulating membrane).	Absent	Absent	Present; ridge-like membrane spirally about body.	Absent	Absent	Absent
Division (character)	Transverse	Transverse	Transverse	Transverse, possibly also longitudinal.	Transverse, possibly also longitudinal.	Transverse
Habitat	Fresh or marine water.	Forammiferous sand.	Parasitic in alimentary canals of fish.	Numerous pathogenic and non-pathogenic varieties.	Two pathogenic and several harmless parasites.	Two pathogenic and one possibly non-pathogenic varieties.
Action of chemicals: Trypsin digestion	Axial filament resistant.	.....	Membrane resistant. Crista and chambers disappear.	.....	Resists for many days.	.....
Bile salts (10 per cent.)	Becomes shadowy, pale, but is not dissolved.	.....	Crista destroyed. Body not attacked.	Complete disintegration.	Complete disintegration.	Easily dissolved.
Saponin (10 per cent.)	Lives 30 minutes; later becomes shadowy, pale, but not dissolved.	.....	Crista becomes fibrillar and then indistinct. Body not affected.	Immobilized in thirty minutes. Broken up in a few hours.	Eventually broken up.	Completely resistant.

infected, and spirochetes are found in large numbers in the blood, but relapses have never been observed.

The pathologic anatomy of the disease presents nothing very characteristic. The spleen is generally enlarged.

Immunity.—Novy and Knapp carried out some important studies on immunity, which not only explain the crisis and the relapse, but establish a basis for the cure and prevention of relapsing fever and related affections. In blood drawn before the onset of an attack, spirochetes will remain in a living motile condition as long as forty days, whereas in blood drawn during the decline of the attack, or after recovery, they have been observed to die out within an hour. In the latter case a powerful specific germicidal body is present. When the parasites are killed or weakened by the germicidal agent, they are taken up by the phagocytes. It usually happens that some of the spirochetes escape this fate, and after an interval become able to multiply, perhaps because of the partial disappearance of the antibodies, perhaps because the spirochetes themselves become immune to the antibodies.[1] Novy and Knapp believe that besides the germicidal agent, an immunizing agent is present, and that the relative amount of these two substances at the close of an attack determines whether or not a relapse will occur. Active immunity follows recovery from infection, and a condition of hyperimmunity can readily be established in rats by repeated injections of blood containing the parasites. The blood of recovered or immunized animals can be used to produce passive immunity, and preventive inoculation can be successfully practised in rats, mice, and monkeys. Animals already infected can be cured, and subsequent relapse prevented by curative doses of blood (about five immunity units per gross body-weight, Novy).

Allied Varieties.—There are probably six or seven distinct diseases caused by organisms similar to the spirochete of European

[1] "Hitherto it was believed that the relapse was due to the survival, in extravascular spaces, of spirochetes which, after the partial destruction or elimination of the specific antibodies, were able again to invade the circulation. In the light of the facts now known it is clear that the relapse is due to the survival of a few individuals which have acquired more or less immunity to the specific germicidal bodies elaborated in the infected animal. As a result, a new 'serum-fast' strain develops, which, in turn, calls for a new antibody. The latter is apparently not as active as the first, or is more unstable, or is more readily eliminated, and hence the continuance of the relapses with this organism." (Novy: Science, 1908, 27, p. 650.)

relapsing fever. A disease of man, proved to be transmitted by the bite of a tick, and known as tick fever,[1] is widely prevalent in equatorial Africa, and has been shown to be due to a spirochete. The parasite of West African tick fever (S. duttoni) differs from S. obermeieri in being about twice as long as the latter, in having wider or looser turns to the spiral, and in possessing diffuse flagella. Fewer organisms are found in the blood of man in tick fever than in relapsing fever, and the effects produced in monkeys and rats by inoculation with S. duttoni are quite different from those caused by S. obermeieri. Another form of African tick fever (East African) is due to a third variety of spirochete, S. kochi.

The parasite (S. carteri) found in a kind of relapsing fever observed in Bombay, India, is apparently different from both of the foregoing (Novy). A number of investigators regard the American case of relapsing fever studied by Novy and Knapp as due to still a fifth spirochete (S. novyi), and Schellack[2] has given exact details of the morphology of the African, American, and European (Russian) forms. Egyptian and Algerian spironema have also been described. Among other organisms of this clan may be mentioned S. anserinum, causing a disease of geese,[3] and possibly identical with S. gallinarum, connected with a very fatal disease of chickens conveyed by tick-bite,[4] and S. theileri, found in small numbers in a benign affection of cattle in South Africa.[5] Spirochetes have also been found in the blood of sheep, horses, and bats, and in the alimentary tract of fish and insects.

Mode of Transmission of Spirochetosis.—In a number of instances spirochetosis is known to be communicated by blood-sucking arthropoda. The African form of relapsing fever was shown in 1905 by Dutton and Todd to be communicated by a species of tick (Ornithodorus moubata). A tick may continue to harbor the parasite for as long as eighteen months after a single meal of infected blood. Especially important is the fact that the spirochete is transmitted to the offspring of the tick, and may even appear in the third generation. The coxal secretion of the ticks is not infective, and it seems to be true that infection is brought

[1] Dutton and Todd: Brit. Med. Jour., 1905, 2, p. 1259.
[2] Schellack: Arb. a. d. k. Gesund., 1907, 27, p. 364.
[3] Sakharoff: Ann. de l'Inst. Past., 1891, 5, p. 569.
[4] Marchoux and Salimbeni: Ann. de l'Inst. Past., 1903, 17, p. 569.
[5] Jour. of Compt. Path. and Therap., 1903, 47, p. 55.

about by contamination of the bite with the infectious excreta of the tick and not by parasites introduced directly by the proboscis. The excreta have been proved to be infective. A rather high temperature (30° to 35° C.) seems to be necessary for the development of the spirochetes within the body of the tick. When the ticks are kept at 15° to 18° C. the spirochetes disappear from the alimentary tract, and the ticks are then unable to transmit infection. On placing them again at 35° C., the ticks become infective. There seems to be no close specific relationship between any particular species of tick and any particular strain of parasite. Indeed, S. duttoni has been transmitted to the rat by the common rat-louse.

There is strong reason for believing that human body-lice are the ordinary agents of transmission of relapsing fever in Europe. The epidemiology of the disease suggests this mode of conveyance, and so does the similarity with typhus fever. Close contact with infected persons and association of the disease with uncleanliness and crowding have long been known to be characteristic features. Inoculation of crushed infected lice will produce the disease in monkeys. Eggs laid twelve to twenty days after infection of the parent lice may contain the spirochete. It now seems to be clear that the bite of the louse can communicate the infection. It is possible that fingers may become contaminated by crushing infected lice and may transmit the infection by scratching. The bedbug may be a mechanical carrier of infection, but does not transmit the disease by its bite, although it may cause infection when crushed and rubbed on the skin. Spirochetosis of fowls is communicated by a tick (Argas persicus) which infests fowls in the warmer parts of the world. The parasite (S. gallinarum), as in human relapsing fever, is transmitted through the egg of the tick to the offspring.

Vincent's Angina (Spironema Vincentis)

In an affection of the mucous membrane of the mouth and throat known variously as "ulceromembranous angina and stomatitis," "Vincent's angina," "pseudomembranous angina," etc., an anaërobic organism, B. fusiformis, has been described by a number of observers as being constantly present.[1] The symptoms and lesions are quite characteristic, and the affection seems rather prevalent, although, from its mild character, it generally passes unnoticed.

[1] Weaver and Tunnicliff: Jour. Amer. Med. Assoc., 1906, 46, p. 481.

The anaërobic, bacillus-like form is a long, slender organism with pointed ends, and is slightly swollen in the middle (fusiform). It is non-motile according to most observers, and does not stain by Gram's method. In fluid media under anaërobic conditions growth is flocculent and whitish. Neither in fluid nor solid media, however, are the cultural features especially characteristic. A foul odor is usually present, but according to most observers there is no formation of gas-bubbles in glucose-agar.

In smear preparations made from the seat of the disease long spirilla are usually associated with the fusiform bacilli; in cultures all attempts to obtain the spirilla or spirochetes without admixture with bacilli have signally failed, hence the relation of these two forms has been the theme of much speculation. Some investigators have supposed that the bacilli and spirilla were distinct organisms, closely associated in some sort of symbiotic relation. Evidence exists, however, in favor of the view that these two forms are simply different phases in the life of one organism.[1]

A similar if not identical organism has been found in noma or gangrenous stomatitis[2] and in some other conditions.[3] Other anaërobic non-spore-forming bacteria associated with spirochetal forms have been occasionally observed in connection with the production of gangrenous and fetid abscesses in various parts of the human body. As an example of this class may be cited B. mortiferus, described by Harris in connection with a fatal case of hepatic abscess in man.[4]

Infectious Jaundice (Leptospira icterohemorrhagiæ)

Epidemics of jaundice have been described now and again during the last hundred and fifty years, one of the earliest being in Minorca in 1745 (Cleghorn). It is not likely that all these epidemics were due to the same cause. One variety of jaundice with fairly definite clinical features has attracted particular attention in recent years. Four cases of this type of jaundice were carefully described by Weil in 1886, and the disease has been frequently known since

[1] Ruth Tunnicliff: Jour. Infect. Dis., 1906, 3, p. 481.

[2] For an excellent historical review of this rare condition see Weaver and Tunnicliff: Jour. Infect. Dis., 1907, 4, p. 8.

[3] See article by Babes on "Spindelförmiger Bazillen" in Kolle and Wassermann's Handbuch, Ergänzungsband 1, p. 271

[4] Harris: Jour. Exper. Med., 1901, 6, p. 519.

as Weil's disease, although different affections have been, very likely, included under this name. A type of jaundice apparently identical with Weil's disease prevailed extensively in the armies during the Great War. Outbreaks of jaundice have occurred in the United States for more than one hundred years and have recently become more widely distributed.[1]

In 1914 Inada and Ido, in Japan, showed the presence of a spirochete in the liver of a guinea-pig inoculated with the blood of a patient suffering from a form of infectious jaundice.[2] The same spirochete was found in the blood and organs of human patients dying from the disease.[3] This organism, now generally known as Leptospira icterohemorrhagiæ, is regarded as the cause of a common Japanese form of infectious jaundice. It is commonly excreted in the urine from the ninth day of the disease or earlier up to the fortieth day. It is somewhat irregular in number of undulations and in size, but averages 8 to 9 μ in length; it can be easily demonstrated by the dark-field method of illumination. "The number of coils is greater in a given length than that of any spirochete hitherto known" (Noguchi). The ends are sharp and are usually hooked in a characteristic fashion. Ito and Matsuzaki[4] succeeded in cultivating the organism by the use of blood-gelatin and blood-agar in various concentrations. Noguchi[5] has also found that the use of a piece of fresh tissue, so advantageous in growing the spirochete of relapsing fever, is not required for the cultivation of this organism, and may be seriously detrimental in a fluid medium. Noguchi recommends two media as equally good for blood-cultures in human cases. One of these is as follows: rabbit serum 1 part + Ringer or 0.9 per cent. sodium chlorid solution 3 parts + citrate rabbit plasma 0.5 part, covered with a thin layer of sterile paraffin oil.

Ido and his associates[6] have shown that the Japanese wild rat frequently harbors the Leptospira icterohemorrhagiæ, and similar findings have been reported in the rats in the United States (New

[1] George Blumer: Jour. Amer. Med. Assoc., 1923, 81, p. 353.

[2] Substantially similar results were obtained a little later by Hübener and Reiter (Deutsche med. Wchnschr., 1915, 41, p. 1275) and by Uhlenhuth and Fromme (Med. Klin., 1915, 11, p. 1202).

[3] Inada, Ido, et al.: Jour. Exper. Med., 1916, 23, p. 377.

[4] Ito and Matsuzaki: Jour. Exper. Med., 1916, 23, p. 557.

[5] Noguchi: Jour. Exper. Med., 1917, 25, p. 755.

[6] Ido, Hoki, Ito, and Wani: Jour. Exper. Med., 1917, 26, p. 341.

York—Noguchi; Nashville—Jobling and Eggstein). The spirochetes have been obtained also in the kidneys of rats caught in certain sectors of the Western battle front where infectious jaundice prevailed. Several proved cases of spirochetal jaundice in North America are on record.[1]

No difference in the character of the spirochetes from these different localities has been demonstrated, but the mortality in the Japanese cases (37 per cent.) is much higher than among the European soldiers (2 to 3 per cent.). The apparently higher virulence in Japan may be connected with the endemic character of the disease in that country and the more frequent transfer of the spirochete from man to man.

The rat is thought by some to be the reservoir of the infective agent, distributing the spirochetes directly or indirectly by means of its urine. The urine or feces of the human patient may also probably serve as the vehicle of infection. This view is borne out by the localization of cases in the trench outbreaks. It is stated that in one sector the cases of jaundice that occurred were almost exclusively in one or two portions of the front line trench, and that as soon as the units were moved out of these particular trenches they ceased to have cases of jaundice, while fresh units previously unaffected commenced almost immediately to develop cases of jaundice when they moved into the trenches. Polluted water is believed by many investigators to play an important part in the spread of the disease.

Inada and his associates have demonstrated the existence of immune bodies in convalescents and in inoculated animals, and have reported encouraging therapeutic results with immune serum. The immune substance persists in the blood of the convalescents for a long time (five and one-half years).

Rat-bite Fever

The bite of a rat is occasionally followed by a series of symptoms indicating a specific infection. The original wound heals, but after a variable incubation period of ten to twenty-two days becomes inflamed and painful. Fever, swelling of the lymph-glands, skin eruptions, and other symptoms occur. The fever is of the relapsing type, with paroxysms at fairly regular intervals, usually about once

[1] Towler and Walker: Jour. Amer. Med. Assoc., 1927, 89, p. 86.

a week, for one to three months. It has been reported from Asia, Europe, and America, and has long been recognized in Japan as a definite febrile disease. Between 50 and 100 cases of this rare human infection have been put on record.

Japanese investigators[1] have found a characteristic spirochete (Spironema morsus-muris) in the swollen local lesions of the skin and the enlarged lymph-glands of patients and also in two instances in the circulating blood. The same spirochete is found in about 3 per cent. of the house rats in Japan. It is not present in healthy mice or guinea-pigs, but if guinea-pigs are bitten by infected rats they develop the disease, and spirochetes are found in their blood. The relapsing character of the fever and the striking clinical effect of the arsenic preparation known as salvarsan upon the disease strengthen the view that the spirochete is the cause of at least a certain number of cases of rat-bite fever. It does not, however, follow that all cases of infection developing after rat-bite are due to this organism. Blake[2] and others have reported instances of apparent streptothrix sepsis following rat bite.

The Micro-organism of Syphilis—Treponema pallidum

Syphilis is essentially an infectious disease, acquired by sexual congress or transmitted by a diseased parent. There are, however, many other modes of infection. Physicians are not infrequently infected in the course of surgical and obstetric practice; wet-nurses of syphilitic children sometimes become infected; and there have been rare cases in which syphilis was communicated by vaccine lymph of human origin. The disease is often congenital, and either parent may be responsible for hereditary transmission. The prevalence of syphilis is much greater than is shown by ordinary hospital and medical records. Routine application of the Wassermann test leads to the recognition of a large number of cases that otherwise could not be diagnosed.[3]

Attention was first directed to the spirochete of syphilis, Treponema pallidum, by Schaudinn and Hoffmann,[4] and their results

[1] Eutaki, Takaki, Taniguchi, and Osumi: Jour. Exper. Med., 1916, 23, p. 249.

[2] Blake: Jour. Exper. Med., 1916, 23, p. 39.

[3] See Public Health Reports, U. S. Public Health Service, 1916, 31, p. 3230.

[4] Schaudinn and Hoffmann: Arb. a. d. k. Gesund., 1905, 22, p. 527; Deut. med. Wchnschr., 1905, 31, p. 711.

were speedily confirmed and extended by Metchnikoff and others. The chief reason why this organism had not been previously discovered by the numerous students of syphilitic lesions seems to be that the spirillum is very difficult to see unstained in the fresh state, and is also exceedingly refractory to stains. The method of staining cover-slip smears found most successful by Schaudinn and Hoffmann consists in the use of Giemsa's eosin solution and azur:

12 parts Giemsa's eosin solution (2.5 c.c. of 1 per cent. eosin; 500 c.c. water).
3 parts azur No. I. (1 : 1000 solution in water).
3 parts azur No. II (0.8 : 1000 solution in water).

The staining mixture should be freshly prepared. Thin films are dried in the air, hardened in absolute alcohol, and immersed in the stain for sixteen to twenty-four hours. They are then washed in water, dried in the air, and examined in cedar oil. By this method the spirilla are stained a pale rose color.

A very simple, rapid, and, withal, effective method of demonstrating the spirochete in smears is based upon the use of India ink. A drop of fluid from the syphilitic lesion is smeared together with a drop of India ink ("Chin-Chin liquid pearl ink") upon a clean slide and allowed to dry. When examined with an immersion lens the organisms are clearly seen against a dark background of carbon particles.

The silver precipitation method, familiar in neurologic work, has been found particularly serviceable for staining the spirochete in tissues, and by its aid the presence of spirochetes in great abundance in congenital syphilis has been demonstrated. Levaditi and Manouélian recommended the following procedure:[1]

1. Fixation of fragments of organs (1 to 2 mm. thick) for twenty-four to forty hours in 10 per cent. formalin.
2. Washing with 96 per cent. alcohol twelve to sixteen hours.
3. Washing with distilled water until fragments no longer float.
4. Impregnation two to three hours at room temperature and four to six hours at about 50° C. in the following bath: Silver nitrate solution, 1 per cent.; pyridin solution (added 10 parts per 100 at moment of use).
5. Very rapid washing in a 10 per cent. pyridin solution.

[1] Levaditi and Manouélian: Comp. rend. Soc. de Biol., 1906, 60, p. 134.

6. Reduction by the following bath: Pyrogallic acid, 4 per cent.; 10 parts per 100 purified acetone, $\frac{5}{8}\frac{8}{8}$, and 15 parts (per total vol.) of pyridin (added at moment of use). The reduction occurs in a few hours.

7. The usual alcohol series; xylol; paraffin; section. The sections may be stained with Unna's blue.

In size the spirilla usually range from 4 μ to 20 μ in length and are very slender, probably rarely reaching 0.5 μ in thickness. The turns in the spiral are close and regular; the number of curves ranges from three to twelve and may reach as high as forty (Goldhorn[1]). There is a fine flagellum at one end (Fig. 119). Movement forward and backward may occur, and also rotation on the axis. The cell is not a perfectly rigid spiral, but is quite flexible. The organism is killed at a temperature between 50° and 55° C. maintained for thirty minutes. Drying is promptly fatal. In undisturbed cultures kept uninterruptedly at 37° C. it may remain alive for as long as one year.

Fig. 119.—The two spirochetes in the center are *Treponema pallidum*, the three others, *Spironema refringens* (Schaudinn and Hoffmann).

Schereschewsky[2] was the first to bring about growth of Treponema pallidum on artificial media. In his experiments, however, growth seems always to have occurred in the presence of other organisms and never in pure culture. Noguchi[3] later succeeded in cultivating several strains of Treponema pallidum through many generations in undoubted pure culture. Great difficulty is experienced in obtaining the first generation from the animal body, and, according to Noguchi, the following conditions are essential: (1) A culture-medium consisting of serum water (1 part serum—sheep, horse, rabbit—and 3 parts distilled water) to which a piece of sterile rabbit tissue—kidney or testicle—is added; (2) strict anaërobic conditions; (3) a temperature of 35° to 37° C. At first the organism

[1] Goldhorn: Jour. Exper. Med., 1906, 8, p. 451.
[2] Schereschewsky: Deut. med. Wchnschr., 1909, 35, p. 835.
[3] Noguchi: Jour. Exper. Med., 1911, 14, p. 99.

will not grow on solid media, but after some transfers growth can be brought about upon serum-water agar, the presence of a bit of rabbit tissue, however, being always necessary. The zone of growth is mostly confined to the neighborhood of the tissue, isolated colonies being seldom formed. In culture the typical morphology is preserved and the power of motility maintained.

Noguchi at first cultivated the spirochete from the testicular lesions of rabbits inoculated with human syphilitic material, but later was also able to cultivate the organism directly from syphilitic lesions in man.[1] Pure cultures so obtained will produce typical lesions in the testicle of the rabbit, and when inoculated into the skin of certain species of monkey lead to lesions resembling the primary syphilitic lesion occurring in man. The blood of monkeys inoculated with pure cultures develops the property of giving a positive Wassermann reaction.

These inoculation experiments with pure cultures forge the last link in the chain of evidence connecting Treponema pallidum with the causation of human syphilis. In the first place, this microorganism, which is well defined and distinguishable from all other known non-pathogenic spirochetes, can be found almost uniformly in the primary lesion or hard chancre. The germ is sometimes localized in the sore in such a way that its presence is not discovered by simple cursory examination, but a thorough search usually reveals it. In the skin eruptions of the so-called secondary stage Treponema is also present, being often demonstrable in great abundance in the papules. The mucous lesions of this stage likewise contain the spirillum, a fact that serves to explain the observed virulence of the saliva. The spirochetes have been found also in the internal organs, such as the liver, kidney, and spleen. Several observers have reported finding Treponema in the circulating blood, although its presence in this situation is naturally difficult to demonstrate. In the tertiary stage the organisms were found at first infrequently, but later observers have been more successful, and there is no doubt that the spirochetes are present in the tertiary lesions also, although in greatly reduced numbers. A number of observers (Finger and Landsteiner,[2] Neisser[3]) have proved that

[1] Noguchi: Jour. Exper. Med., 1912, 15, p. 90.

[2] Finger and Landsteiner: Sitz-Ber. Akad. Wiss., 1905, 1906.

[3] Neisser: Deut. med. Wchnschr., 1904, 30, pp. 1369, 1431; 1906, 32, pp. 1, 49, 97.

material from tertiary lesions can produce infection, although many of the attempts result in failure.

Convincing testimony in favor of causal relationship is afforded by the presence of Treponema pallidum in the lesions of congenital syphilis (Fig. 120). The microbe is found in large numbers and in pure culture in the internal organs of syphilitic infants and under conditions where secondary infection would seem to be excluded. In the liver especially the spirilla are very abundant. The organism

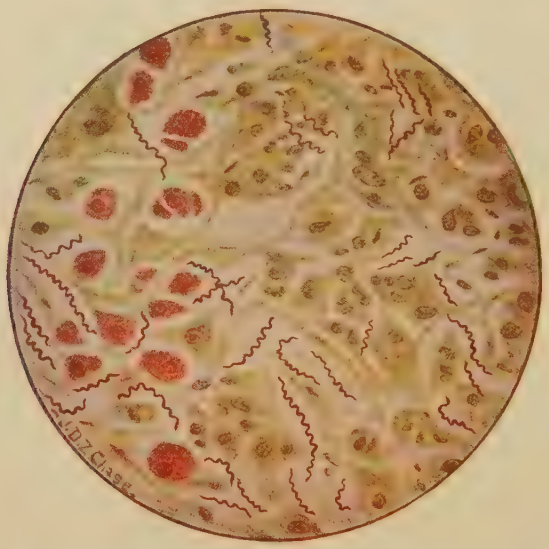


Fig. 120.—Treponema pallidum in congenital syphilis. × 1000. Levaditi (Sobernheim, in Kolle and Wassermann).

seems to be primarily an intracellular parasite, which is especially apt to attack glandular epithelium.

Still further indication of the pathogenicity of this micro-organism is given by its frequent occurrence in the syphilitic lesions of monkeys that have been successfully inoculated with syphilitic material from man or from another monkey. Metchnikoff and Roux[1] determined the presence of Treponema in 70 per cent. of such cases that they examined. As in the lesions of congenital syphilis, the micro-organism is found in the infected monkeys in pure culture.

[1] Metchnikoff and Roux: Ann. de l'Inst. Past., 1903, 17, p. 809; 1904, 18, pp. 1, 657; 1905, 19, p. 673.

The earlier attempts to inoculate the lower animals with syphilitic virus gave variable and conflicting results. Metchnikoff and Roux were the first to show that syphilis could be successfully and uniformly communicated to monkeys, and that the material from the lesions so produced could be used for successive reinoculations. The technic of inoculation is important. Subcutaneous, intraperitoneal, and intravenous inoculations, even of the most virulent material, are without effect, but cutaneous inoculation, particularly upon the eyebrows and genitals, is usually followed by typical primary lesions. Positive results are almost invariably obtained in these situations when the virus is freshly procured and when deep scarification is practised. The period of incubation varies from fifteen to fifty days. Practically all species of monkeys are susceptible. Material from the primary and secondary lesions and from congenital syphilis has been found capable of producing infection. In the higher monkeys (chimpanzees and gibbons) the primary effects have been followed by typical secondary syphilitic manifestations after a few weeks. In simian syphilis spirochetes have been found distributed in the same manner as in man, although in less abundance, as corresponds with the lower susceptibility of the monkey tribe.

Bertarelli,[1] Schucht,[2] and others have been able to produce inoculation syphilis of the iris and cornea in rabbits. The average period of incubation in these animals is about twenty-nine days. The process remains localized, and none of the rabbits showed lesions in the internal organs. Treponema pallidum was found in large numbers in the affected parts. Uhlenhuth and Mulzer[3] have produced typical syphilitic orchitis in rabbits, a lesion that has proved a convenient source of spirochetes in practically pure culture.

The Wassermann Reaction.—Wassermann and his collaborators[4] have demonstrated by the method of complement fixation (p. 177) the presence of specific antibodies in the bodies of syphilitic individuals and in the serum of monkeys treated with syphilitic virus.

[1] Bertarelli: Centralbl. f. Bakt., I, Orig., 1906, 41, p. 320.

[2] Schucht: Münch. med. Wchnschr., 1907, 54, p. 110.

[3] Uhlenhuth and Mulzer: Arb. a. d. k. Gesund., 1909–10, 33, p. 183.

[4] Wassermann et al.: Deut. med. Wchnsch., 1906, 32, pp. 745, 1769; Ztschr. f. Hyg., 1907, 55, p. 451.

The Wassermann reaction has been extensively used for the diagnosis of syphilis. The method is based on the fact that complement fixation occurs when an antigen and its specific antibody are mixed in the presence of free complement. Under suitable conditions all the complement in a serum may be used up or fixed, and the further addition of another antigen and antibody will not be followed by a lytic action. If, for example, a mixture of red blood-corpuscles and the corresponding inactivated hemolytic serum be added to a fluid containing free complement, hemolysis will occur; but if the complement is already fixed, the "hemolytic system" is not complete and no hemolysis takes place. An extract from a syphilitic organ, such as the spleen of a syphilitic fetus, contains the antigen, and when this is mixed with the inactivated serum of a syphilitic patient (antibody) in the presence of complement the complement is fixed. Now when such a mixture is added to an emulsion of red blood-cells, together with the appropriate inactivated hemolytic serum, no hemolysis will result, because the complement was fixed by the first combination of antigen and amboceptor. If, on the other hand, the serum is taken from a patient not suffering from syphilis the complement will not be anchored (since neither antigen alone nor amboceptor alone can fix complement), and when hemocytes and hemolytic serum are added hemolysis will occur.

The substances usually employed in the test are as follows:

(*a*) *Hemolytic serum*, prepared by injecting a rabbit with the red blood corpuscles of the sheep. The corpuscles are first washed in physiological salt solution and then a 5 per cent. emulsion made in salt solution. An increasing amount of this emulsion—5, 10, 15 c.c., etc.—is injected intraperitoneally or intravenously into the rabbit at intervals of five or six days. Three or four injections are usually sufficient. The rabbit may be bled nine or ten days after the last injection and the serum drawn off from the clot with the usual aseptic precautions. The serum should be inactivated by heating for half an hour at 55° to 56° C.

(*b*) *Sheep corpuscles* may be obtained by receiving the blood into a sodium citrate solution which prevents clotting, or by defibrinating the blood by whipping or shaking with glass beads. Before use the corpuscles must be thoroughly washed in salt solution, and then mixed with sterile salt solution to form a 5 per cent.

emulsion. A mixture of (*b*) with the inactivated hemolytic serum (*a*) contains of course amboceptor and antigen, but no complement, and hence, hemolysis does not occur. Addition of complement will complete the "system" and evoke hemolysis.

(*c*) The *complement* for the Wassermann reaction is usually obtained from the guinea-pig, since the guinea-pig complement is active, readily fixed, and does not deteriorate readily. Blood is drawn from a normal animal and the serum separated by centrifugalizing after the clot is formed. When kept on ice the serum remains active and may be used for as long as three days.

(*d*) The *antigen* was first prepared by Wassermann and others from syphilitic organs, either directly by salt solution or by evaporating an alcoholic extract. The theoretically disturbing discovery has since been made that the extract of normal organs of man and other animals contains an antigen, which gives practically as reliable results as the antigen of specific syphilitic tissues. This is not the place to discuss the theoretical significance of this fact; in practice the circumstance has been found convenient.

Liver or heart tissue (normal or syphilitic) will furnish a satisfactory antigen. The tissue is cut into small pieces and macerated in a sealed jar with four volumes of 95 per cent. alcohol at room temperature for six to seven weeks. The alcohol is then filtered through paper and a second extract made with four volumes of absolute alcohol at 37° C. for four days with daily shaking. The second extract is added to the first and the mixture evaporated to dryness. The ether-soluble portions are then removed by treatment with a small amount of ether. The ether is then added to ten times its volume of C. P. acetone. The precipitate that forms contains the antigen. It may be dissolved in ether or alcohol and preserved for a long time, at least a year (Noguchi).

"A standard antigen for Noguchi's method for the diagnosis of syphilis may be prepared as follows: As a stock solution make an ethereal or alcoholic 3 per cent. solution of an acetone-insoluble fraction of the liver or heart. From this an emulsion is made by shaking one volume of the stock solution with nine volumes of a 0.9 per cent. sodium chloride solution. This emulsion is tested for its properties. If it is hemolytic or anticomplementary, in the dose of 0.4 c.c., it is unsuitable. When the emulsion is found to be non-hemolytic and non-anticomplementary, it is tested for

its antigenic strength. If it produces complete inhibition of hemolysis with one unit[1] of syphilitic antibody, in doses of 0.02 c.c. or less, it is suitable. In the fixation test 0.1 c.c. of such an emulsion is to be used, thus employing more than five times the minimal antigen dose. So far as our experience goes, the use of several antigen doses does not cause a non-specific fixation and is not unduly sensitive."[2]

(*e*) The *serum to be tested* is best obtained from the arm vein of the patient in the way that blood is drawn for blood-cultures. An amount of blood sufficient to furnish 1 c.c. of serum should be removed. Before use the serum should be inactivated, preferably at 54° to 55° C. for thirty minutes.

The test must be carefully controlled at every point. In the first place it is necessary to know whether the hemolytic system works properly. This may be determined with the following mixture: (*a*) 1 c.c. of 5 per cent. emulsion of sheep corpuscles, (*b*) 0.1 c.c. complement, (*c*) 2 units hemolytic serum. This mixture should be made up to 5 c.c. with sterile salt solution. When incubated at 37° C. for one hour it should show complete hemolysis both in the presence and absence of antigen.[3]

In each test of a suspected serum a control should be carried out with normal serum and with known syphilitic serum (0.2 c.c.), preferably in two sets of tubes, one with and one without antigen. A positive result is indicated by suspension of hemolysis in the tube with control syphilis serum and antigen, and in the tube with suspected serum and antigen. All the others give hemolysis in one hour at 37° C.

[1] One unit is that amount of amboceptor (inactivated hemolytic serum) (*a*) which is just sufficient to bring about hemolysis of 1 c.c. of 5 per cent. emulsion. Hence, the hemolytic serum must first be titrated by using graded amounts of serum. The presence of too large an amount of amboceptor interferes with the reaction.

[2] Noguchi and Bronfenbrenner: Jour. Exper. Med., 1911, 13, p. 66.

[3] The amount of antigen necessary in the tests must be determined by mixing graded quantities of the antigen with one set of tubes, containing 0.1 c.c. complement and 0.2 c.c. inactivated normal serum, and another set with complement and known syphilitic serum. Allow these mixtures to stand for an hour and then add red corpuscles (*b*) and hemolytic serum (*a*). That amount of antigen which will permit complete hemolysis to occur with the normal serum, but will cause complete inhibition with the syphilitic serum, is the suitable amount to use in the tests.

The Kahn Precipitation Test.—The fact that the serum of syphilitic patients shows an increased power to flocculate or precipitate colloidal matter has been made the basis of several specific flocculation tests. The Kahn precipitation test has come into wide use in the United States. It possesses the advantages of simplicity, cheapness of the necessary ingredients and, perhaps, somewhat greater sensitiveness than the Wassermann reaction.

The technic as described by Kahn[1] is as follows:

I. Glassware and Apparatus Required for the Test

All glassware employed in the test must be chemically clean and neutral.

1. *Test tubes:* Standard antigen dilution tubes are 5.5 cm. in length and 1.5 cm in diameter.

Standard tubes for performing the test are 7.5 cm. in length and 1 cm. in diameter.

2. *Test tube racks:* Racks should be of such construction as to permit vigorous shaking of the tubes.

3. *Pipets:* 10 c.c. pipets marked in 0.1 c.c. quantities. 1 c.c. pipets marked in 0.01 c.c. quantities. 0.2 c.c. pipets marked in 0.001 c.c. quantities.

4. *Shaking machine:* May be of any construction which will hold the test tube racks employed.

5. *Inactivating bath* (56° C.) as well as centrifuge and centrifuge tubes may be of any make which will be found convenient in the particular laboratory.

II. Reagents Employed in the Test

The three ingredients entering into the test are (1) Antigen, (2) Serum, and (3) Physiologic Salt Solution.

1. ANTIGEN

Method of Preparation.—The method of antigen preparation has been standardized with a view to eliminating several of the variable elements inherent in the method previously used.

The unit amount of powdered beef heart used for preparing antigen is 25 gms. and it is always extracted in a 250 c.c. Erlenmeyer flask. The ether extraction consists of four ether "washings" of the powdered muscle at ten minute intervals with 100, 75, 75 and 75 c.c. ether, respectively. The subsequent alcohol extraction is carried out for three days at room temperature (21° C.). Twenty-five gms. of powdered beef heart will yield about 75 c.c. antigen. If the preparation of larger amounts is desired, as many 25 gm. quantities are employed as needed.

Powdered Heart Muscle.—About 400 gms. of heart muscle are cut out from at least three fresh beef hearts and passed four times through a meat grinder.

[1] Kahn: Am. Jour. Pub. Health, 1924, 14, p. 498; see also Kahn: "Serum Diagnosis of Syphilis by Precipitation," Baltimore, 1925, p. 237.

The ground material is spread into a thin layer on a porcelain platter or glass plate and dried by means of one or two revolving fans. After six or eight hours, when the exposed surface is relatively dry, the material is turned over and drying continued over night. When the layer of beef heart is in the form of a dry plate, it is broken up into small pieces and drying continued until the material is brittle and easily breakable. The material is now ground into powder form by means of a mortar or coffee grinder which is used for no other purpose.

Powdered beef heart is now obtainable on the market from the Digestive Ferments Company, Detroit. It is prepared essentially as outlined above, except on a large scale—as many as seventy-five beef hearts entering into the preparation of a given lot. This assures a higher degree of uniformity than can be obtained with three beef hearts. Because of this uniformity element, the market product is used in this laboratory almost exclusively.

Extraction of Powdered Muscle with Ether.—Twenty-five gm. of powdered beef heart are placed in a 250 c.c. Erlenmeyer flask and 100 c.c. ether (anesthesia) added. The flask is shaken from time to time for an interval of ten minutes after which the ether is filtered off. The filtration process may be hastened somewhat by applying gentle pressure to the beef heart by means of a spatula. Filtration is completed when pressure with the spatula does not cause drops of ether to pass through the funnel.

The moist beef heart is now returned to the original 250 c.c. extraction flask. This may be done by first transferring the beef heart from the funnel to a sheet of white paper and breaking the material up with a spatula into pieces small enough for the mouth of the flask. Seventy-five c.c. ether are now added and the mixture again shaken for a ten-minute interval and filtered as above.

After the second filtration of ether, the beef heart is transferred to the flask for a third time and 75 c.c. ether again added. The mixture is shaken for a ten-minute interval and again filtered as described above.

The moist beef heart is now transferred for the fourth and last time to the flask and 75 c.c. ether added, the mixture shaken for ten minutes and filtered. After gentle pressure with a spatula does not cause drops of ether to pass through the funnel—the end-point employed in each of the four ether filtrations—the beef heart is transferred to a sheet of white paper and dried either at room temperature or at 37° C. The drying usually requires from ten to fifteen minutes. When no ether odor is detectable, the beef heart is ready for extraction with alcohol. This extraction may be carried out in the same flask used for the ether extractions, provided the flask is entirely freed from ether odor.

Extraction of Powdered Muscle with Alcohol.—The ether extraction being completed, the dry powdered muscle is weighed and placed in a 250 c.c. flask. Usually there will be 23 gms. or less of the powder due to the loss during the ether extraction. Five c.c. of 95 per cent. alcohol are added per gm. of powder. The flask is shaken for ten minutes and extraction allowed to continue at room temperature (21° C.) for three days without shaking. At the end of this period, the mixture is shaken for five minutes and filtered. The filtrate is kept in the dark at room temperature as stock antigen solution.

Cholesterinization of Alcoholic Extract.—A given amount of alcoholic extract —likely to be used in a month or two—is cholesterinized by adding 6 mg. of chemically pure cholesterin per c.c. of extract. The cholesterin is dissolved by rotating the flask in a water bath at 37° C. When all the cholesterin has

been dissolved, the antigen is filtered to remove impurities and allowed to stand one day. It is then ready to be titrated or standardized for the test.

It is well to emphasize in connection with antigen preparation that the ether and alcohol employed should be of high purity and that the latter should be 95 per cent. At one time we unknowingly used 80 instead of 95 per cent. alcohol with misleading results.

Tinfoil-covered corks have been found most satisfactory as stoppers for flasks used in antigen preparation and storing. Rubber as well as cork stoppers give off soluble elements into the alcohol which modify the final product.

Method of Antigen Titration.—The aim of the titration of antigen for this test is to find the minimum amount of physiologic salt solution to use with antigen which will result in an antigen-salt solution precipitate that is soluble on further addition of salt solution. The titration is carried out in the presence of salt solution and not in the presence of serum, although the latter may be used as a check on the titration, if desired.

One c.c. amounts of cholesterinized antigen are added to each of five standard antigen dilution tubes (5.5 cm. length and 1.5 cm. diameter). To five similar tubes are added the following amounts of physiologic salt solution, respectively: 0.8, 0.9, 1.0, 1.1 and 1.2 c.c. Each salt solution tube is emptied into a given antigen tube and, without waiting to drain the salt solution, the mixture is immediately poured back and forth five or six times to permit thorough mixing. Each of the five antigen dilutions will show the presence of a definite precipitate. The character of these precipitates, however, will vary according to the quantity of salt solution used. Some of the precipitates will be found to be stable and will not dissolve when mixed with salt solution; other precipitates again will readily dissolve in salt solution.

The solubility in salt solution of each of the five antigen dilution precipitates is tested as follows: 0.05, 0.025 and 0.0125 c.c. amounts, respectively, of each antigen dilution are pipetted with a 0.2 c.c. pipette graduated in 0.001 c.c. into three tubes (7.5 cm. in length and 1 cm. diameter). These small quantities are pipetted in each case to the bottom of the tubes. 0.15 c.c. quantities of physiologic salt solution are now added to each tube. The rack is shaken vigorously for two minutes after which 0.5 c.c. salt solution is added to each tube and observation made as to whether or not the original antigen dilution precipitate has gone back into solution. The antigen dilution tube containing the smallest amount of salt solution in proportion to antigen, having a precipitate which goes back into solution in salt solution, as shown by this three tube test, represents the endpoint of this titration and determines the proportion in which antigen is to be mixed with salt solution in the performance of the tests.

The accompanying table gives an outline of a typical antigen titration.

TABLE 1

TYPICAL ANTIGEN TITRATION FOR TEST WITH SERUM

Antigen Dilution Series	1	2	3	4	5
Antigen + Salt Solution c.c.	1 .8	1 .9	1 1.0	1 1.1	1 1.2
Result of Dilution	Heavy precipitate in each antigen dilution				
Scheme Used in Testing Solubility of Precipitate in Each Antigen Dilution	Tube No. 1 2 3 Antigen Dilution[1] c.c. .05 .025 .0125 Salt Solution c.c. .15 .15 .15 Tubes are shaken 2 minutes and 0.5 c.c. salt solution added to each. All are observed for precipitates.				
Solubility of Precipitate as Determined by Three-tube Test	Precipitate not soluble	Precipitate not soluble	Precipitate soluble	Precipitate soluble	Precipitate soluble
Standard Antigen Dilution			Antigen + minimum amount of salt solution giving precipitate which dissolves in salt solution		

[1] Each antigen dilution is allowed to stand 30 minutes after mixing antigen and salt solution before solubility test is made.

2. SERUMS

The serums are separated from the clots by centrifugation in the usual manner, pipetted off and inactivated for 30 minutes at 56° C. The main point regarding serums is that they be free from red cells, fibrin and particles of any kind. No difficulty is encountered in this test with milky or chylous serums or with moderately hemolyzed specimens. Only such hemolyzed specimens which are not fit for a Wassermann test are not fit for a precipitation test.

3. PHYSIOLOGIC SALT SOLUTION

Salt solution is prepared by dissolving 8.5 gm. of chemically pure sodium chloride per liter of distilled water and filtering. Although sterility of this solution is not essential, the same type of chemical cleanliness usually employed in quantitative chemical work is required.

III. THE ROUTINE TEST WITH SERUM

The routine test consists of three tubes containing three different proportions of serum and antigen dilution in accordance with the following outline:

Tube No.	1	2	3
Serum: Antigen Dilution	3 : 1	6 : 1	12 : 1
Antigen Dilution c.c.	.05	.025	.0125
Serum c.c.	.15	.15	.15

Preparation of Standard Antigen Dilution.—It is well when carrying out a number of tests to inactivate the serums and set up and number the tubes before diluting antigen with salt solution for the tests. The antigen dilution is so

standardized as to necessitate its use within a half hour after mixing antigen with salt solution. In this laboratory, with over 200 routine tests per day, enough antigen dilution for 60 or 80 tests is usually prepared at one time—two experienced workers pipetting antigen dilution and serum for about 80 tests in less than twenty minutes.

One c.c. antigen diluted with one c.c. salt solution gives sufficient antigen dilution for about 18 tests. Two or three c.c. antigen may be diluted with corresponding amounts of salt solution by utilizing the same standard antigen dilution tubes.

Procedure: One c.c. antigen is measured into an antigen dilution tube. An amount of salt solution usually approximating that of the antigen, according to the titer of the antigen, is measured into a similar tube. The salt solution is poured into the antigen and, without waiting to drain the tube, the mixture is immediately poured back and forth five or six times to insure thorough mixing. For uniformity, this antigen dilution is permitted to stand ten minutes at room temperature before pipetting.

As a preliminary control of the antigen dilution, 0.05 c.c. is measured into a test tube, 1 c.c. salt solution added and the tube shaken vigorously for ten or fifteen seconds. The mixture should appear opalescent.

Pipetting of Antigen Dilution.—The antigen dilution is always pipetted to the bottom of the tubes. The 0.05 c.c. amounts may be pipetted with a 1 c.c. pipet in which 0.05 c.c. graduations are indicated with a wax pencil. For the 0.025 and 0.0125 c.c. amounts of antigen dilution, 0.2 c.c. pipets are employed and the proper markings may also be indicated with a wax pencil. The antigen dilution should be mixed frequently during the pipetting period to assure a uniform mixture. Due to the possibility of evaporation of the small amounts of antigen dilution used in the test, it is necessary to pipet the serum within several minutes after the antigen dilution has been pipetted. If a worker desires to run forty precipitation tests, for example, it is better to pipet antigen dilution and serums for ten tests at a time, than to pipet the antigen dilution for all the tests first and then follow with the serums.

Pipetting of Serum.—The 0.15 c.c. amounts of each serum are added to the antigen dilution by means of a 1 c.c. pipet graduated in 0.01 c.c. In pipetting these amounts of serum it is not necessary to lower the pipet to the bottom of the tube. As soon as the serums have been added for ten tests, or less, the rack is shaken sufficiently to insure thorough mixing of the serum with antigen dilution.

Shaking of Tests.—After the serums have been mixed with the antigen dilution, the tests are shaken for a two minute interval. A shaking machine is of the utmost importance for this purpose, particularly in the examination of comparatively large numbers of specimens at a given time.

Effect of Incubation.—Although the final results may be read immediately after the shaking period, different workers have observed that a 15 minute incubation period in the water bath at 37° C. produces sufficient clumping of the precipitates to make the reading of the results easier, particularly in the case of weak reactions. In studying the effect of 15 minute incubation on this test, no tendency for false positive reactions has been observed, and on the basis of easier reading we consider the employment of this incubation period of some advantage.

Addition of Salt Solution and Reading of Results.—After the shaking of the tests as well as after the 15 minute incubation period, if employed, the serum-

antigen mixtures appear uniformly cloudy. In order to render the negative reactions clear and thus simplify the reading of results, 0.5 c.c. salt solution is added to each tube. The tests should be read immediately after the addition of salt solution as an occasional weak precipitate may go back into solution on standing.

Readings are best made in front of a window with a darkened background. The negative serums appear opalescent and readily distinguishable without lifting the tubes from the racks. The strongly positive serums show heavy precipitates which are also easily read directly.

Only the tubes showing weak reactions need to be removed from the rack and examined individually. Each tube is lifted from the rack above the eye level, slanted to spread the fluid into a thin layer and examined for a precipitate.

In this laboratory, precipitation tests are read at night with the same ease as during the day. Light is furnished by a 300-watt daylight bulb in an overhead indirect fixture.

Note: If it is desired to make a check reading of the results at some later period, it is well to keep the tests at icebox temperature. Practically all positive reactions will be slightly stronger at the second reading. Occasionally, however, even at icebox temperature, a serum which gave a −, +, ++, at the first reading may be found to be negative during the second reading. We have observed these reversible precipitation reactions especially in early primary and in highly treated cases. The reading made immediately after the addition of 0.5 c.c. salt solution to each tube is the standard reading and is always the one reported.

The Control System.—1. Antigen Control. When pipetting antigen dilution for a series of tests, the last set-up of three regular antigen amounts receives 0.15 c.c. salt solution instead of serum and is read with the regular tests. All three tubes should show freedom from a precipitate.

2. Serum Controls. In the case of each positive reaction, the serum used in the test is examined for particles that might give the appearance of a specific precipitate.

3. Positive and Negative Controls. One or more such controls are included with each series of tests.

Interpretation of Results.—A definite precipitate suspended in a clear medium is considered a complete reaction and is read four plus. Proportionally weaker reactions are read three, two and one plus, respectively. The final result in each test is the average finding of the three tubes. Thus, if the precipitation reaction is four plus in each of the three tubes, the final result is four plus. If the reaction is −, +++, ++++, the final result is two plus. A number of typical reactions with this method and the final result in each case, are illustrated in Table 2 [p. 523].

Comparative records of Kahn and Wassermann tests in the laboratories of the Michigan State Board of Health on over 174,000 specimens showed a close agreement in the results; when applied to the serum of syphilitic patients under treatment the Kahn test appeared somewhat more sensitive.[1]

[1] Kahn, Kendrick and Landau: Jour. Amer. Med. Assoc., 1927, 89, p. 84.

TABLE 2

INTERPRETATION OF RESULTS OF ROUTINE TEST WITH SERUM

Tube No.		1	2	3	Average of reaction in the three tubes = final result
Serum: Antigen Dilution		3 : 1	6 : 1	12 : 1	
Antigen Dilution c.c.		.05	.025	.0125	
Serum Undiluted, c.c.		.15	.15	.15	
	Reaction No.				
	1	++++	++++	++++	++++
	2	+++	++++	++++	
	3	++	++++	++++	
Some	4	+	++++	++++	+++
	5	−	++++	++++	
	6	−	+++	++++	
Typical	7	−	++	++++	++
	8	−	+	++++	
	9	−	−	++++	
Reactions	10	−	−	+++	+
	11	−	+	++	
	12	−	±	++	
	13	−	−	++	±
	14	−	±	+	

YAWS

Castellani[1] has reported the presence of spirochetes in a disease occurring in several tropical countries, and known in the West Indies as yaws. The disease resembles syphilis, and by some writers has been regarded as identical with it. The mode of transmission of yaws has yet to be established; it is uncertain whether biting insects have any part in it. The spirochete Treponema pertenue found in yaws is long and very slender; there may be as many as twelve spirals. The Romanowsky staining method or some modification of it is said to give the best results. Vom dem Borne[2] has found Tre. pertenue in 73 out of 76 cases in which the young intact papules of the disease were examined. Material obtained from persons suffering from yaws and apparently containing

[1] Castellani: Brit. Med. Jour., 1905, 2, p. 1280.

[2] Vom dem Borne: Jour. Trop. Med., 1907, 10, p. 345.

Tre. pertenue as the sole micro-organism is infectious for monkeys, and the spirochete is practically always present in the unbroken eruptive lesions and is often found in the spleen and lymph-nodes. Castellani[1] has obtained evidence of the existence of specific yaws antibodies and antigens which are different from syphilis antibodies and antigens. Ashburn and Craig[2] emphasize the morphologic resemblance of Tre. pertenue to Tre. pallidum, but nevertheless conclude that Tre. pertenue can be differentiated by inoculation experiments and that it is the cause of yaws.

Nichols[3] has produced lesions in the testicles of rabbits which are analogous with those of syphilis, and contain Treponema pertenue in large numbers in practically pure culture.

Yellow Fever (Leptospira Icteroides)

Yellow fever is primarily a disease of the tropics, but is occasionally introduced into the temperate zone, where it can flourish prodigiously for a time until checked by the occurrence of frost. The infectious character of yellow fever has long been manifest, and for many years the belief prevailed among many physicians that the disease could be disseminated through the atmosphere. Others, however, maintained that it was directly contagious, or, at any rate, could be communicated through clothing, bedding, and the like. The confusion that existed as to modes of transmission was first dispelled by the experimental work of Reed, Carroll, Agramonte, and Lazear.[4] These investigators showed that the bite of a particular species of mosquito (Aëdes ægypti, formerly known as Stegomyia calopus) (Fig. 121) could communicate the disease, thus furnishing proof of a theory advanced by Finlay in 1881,[5] and earlier by Nott.[6] The evidence in favor of mosquito transmission is briefly as follows:

Men volunteering to submit themselves to experimentation were first subjected to a period of strict quarantine and then exposed to the bite of mosquitoes that had previously bitten yellow fever patients. They then returned to quarantine. In the

[1] Castellani: Jour. of Hyg., 1907, 7, p. 558.

[2] Ashburn and Craig: Philippine Jour. of Sci., B. 1907, 2, p. 441.

[3] Nichols: Jour. Exper. Med., 1910, 12, p. 616.

[4] Reed, Carroll, Agramonte, and Lazear: Philippine Med. Jour., 1900, 6, p. 790; Rep. and Papers, Amer. Pub. Health Assoc., 1900, 26, p. 37.

[5] Finlay: Jour. Amer. Med. Assoc., 1901, 37, p. 1387.

[6] Nott: New Orleans Med. and Surg. Jour., 1848, 4, p. 580.

majority of instances an attack of yellow fever followed the mosquito bite in about five days. Further experiments showed that the subcutaneous injection of 0.5 c.c. of blood drawn directly from the veins of a yellow fever patient on the first, second, or third day of the disease would cause an attack of yellow fever. The blood-serum is still infectious after it has been passed through a Berkefeld filter (Carroll). It was also found that a certain period—at least twelve days—must elapse after a mosquito had bitten a yellow fever patient before the insect could communicate the disease. This is taken to indicate that the virus of yellow fever passes through a developmental cycle in the body of the mosquito. A mosquito once infected remains so during life. Hence a mosquito infected in the autumn of one year may carry over the infection to the following year. Some observers have claimed that adult infected mosquitoes can transmit infections to the ova and larvæ, but the weight of evidence is against this view. The ability to transmit yellow fever is limited, so far as known, to one particular species, *Aëdes ægypti*, having a world-wide distribution, but restricted in its breeding to warm climates (43° S. to 43° N. latitude).

Fig. 121.—Aëdes aegypti, female (Boyce, after Newstead).

Many experiments have been made to determine whether yellow fever can be communicated in any other way than by the bite of the mosquito. Volunteer experimenters have lived and slept in closest contact with clothing and bedding used by yellow fever patients. "One of them slept with his head upon a towel soiled with blood drawn from a patient in the first day of the disease, and which was shown by inoculation to be capable of infecting" (Carroll). All of these attempts to produce infection from the yellow

fever patient or his belongings without intervention of the mosquito have failed, and there is today no evidence of natural transmission other than through the medium of the mosquito.

One of the most satisfactory methods yet found of combating yellow fever is to attack the mosquito host. *Aëdes* is seldom found far from human habitation, and breeds by preference in artificial collections of water, especially in rain-water cisterns and barrels. Destruction of larvæ with oil, and of the adult insects by sulfur fumigation, combined with the elimination or screening of possible breeding-places, are measures that have been employed with great success. Mosquitoes should also be barred from all possible avenues of approach to yellow-fever patients.

In Havana, Cuba, during the year 1900–01, "when the sanitary authorities were putting forth every effort known at that time to sanitary science in order to control the march of the disease," the yellow-fever cases numbered 1240 and the deaths 305; in the following year, 1901–02, when "yellow fever was fought on the theory that the specific agent of this disease is transmitted solely by means of the bites of infected mosquitoes," there were 31 cases and 6 deaths.[1] In the years 1902–04 and 1909–11 there were no deaths from yellow fever in Havana: the small outbreak occurring in 1905–08 was quickly checked. Similar success has been obtained in Guayaquil, long notorious as a port heavily infested with yellow fever. The antimosquito campaign carried on by the International Health Board in co-operation with the local authorities has resulted in completely freeing Guayaquil and the surrounding territory from the plague that has long ravaged it. It does not seem too much to hope that by the application of such measures yellow fever may be entirely eliminated from the face of the earth.

A spirochetal organism (Leptospira) was detected in 1918 by Noguchi in the blood and liver of yellow-fever cases in Guayaquil.[2] Certain reasons have been advanced for regarding Leptospira icteroides as the cause of yellow fever. It has been found present in clinically characteristic human cases of yellow fever in Guayaquil and in Mexico, and in guinea-pigs inoculated with blood from yellow fever patients. Pure cultures were obtained by Noguchi and found

[1] Reed: Jour. of Hyg., 1902, 2, p. 101.

[2] H. Noguchi: Jour. Exper. Med., 1919, 28, pp. 547, 565, 585; 30, pp. 1, 9, 13, 87, 95, 401; 1920, 31, p. 159; 32, pp. 601, 627.

to produce in guinea-pigs characteristic symptoms and lesions. From infected guinea-pigs the infection could be transmitted to normal guinea-pigs by the bite of the Aëdes mosquito. The Leptospira organism conforms in its characters to all the previously determined features of the yellow fever virus, such as passage through Berkefeld filters, rapid destruction at 55° C. and in the presence of bacteria of decomposition, mosquito transmission with a necessary incubation period, and failure to grow under ordinary conditions of bacterial cultivation. Finally, a convincing demonstration of the causal relation of Leptospira icteroides to yellow fever was believed to be achieved in protecting against yellow fever by use of a vaccine made from pure cultures of the Leptospira, and by encouraging results obtained in the cure of yellow fever cases by use of an antiserum prepared from this organism. These immunological data, combined with the other evidence elicited by the brilliant studies of Noguchi and his associates, have been thought to render it highly probable that Leptospira icteroides is the causative agent of yellow fever.

Serious objections, however, have been raised to this view. Perhaps the most weighty of these is the close relationship of Leptospira icteroides and L. icterohemorrhagiæ, the cause of Weil's disease. Cross-immunity tests show no difference whatever between these two organisms. Blood from yellow fever patients fails to give a positive Pfeiffer reaction with either L. icteroides or L. icterohemorrhagiæ (Sellards). Aëdes mosquitoes are easily infected with the virus of yellow fever, but are infected with difficulty if at all with L. icteroides. Guinea-pigs are easily infected with L. icteroides, but are difficult to infect with the virus of yellow fever. Weil's disease is easily transmitted by the urine of a patient; the yellow fever virus apparently does not leave the body by this route. For these and other reasons many bacteriologists consider the etiological relation of L. icteroides to yellow fever still an open question.

CHAPTER XXVIII

THE PATHOGENIC TRICHOMYCETES

The organisms included under this head are filamentous forms and possess many characters relating them to the group of true molds (Hyphomycetes) rather than to the bacteria proper (Schizomycetes). They may be said perhaps to stand in an intermediate position between the bacteria and the higher fungi. All have a more complicated cycle of development than has been observed in any of the true bacteria. On the other hand, they are distinguished from the higher fungi by the slenderness of their filaments and their more simple life-history. Among the so-called Trichomycetes themselves great confusion has prevailed both in classification and in nomenclature, and there is still far from being any universal agreement concerning the subdivisions of the group and their designations. The following table will serve to make clear the nature of several of the better marked divisions under what seem to the writer the most appropriate generic names:

Trichomycetes	Leptothrix	No branching.
	Cladothrix	"False" branching.[1]
	Nocardia[2]	True branching. Reproductive elements—spores—observed.
	Actinomyces	True branching. No spores observed.

Leptothrix Mycoses

A number of writers have reported cases of suppurative processes due to organisms of this class (B. Fränkel,[3] Arustamoff,[4]

[1] The terminal cell, in dividing, pushes the product of the previous division to one side. Thus there are two terminal cells lying side by side, and as each goes on dividing, an appearance of branching is produced.

[2] Sometimes termed Streptothrix, a name also applied by some writers to the whole group of Trichomycetes. According to the rules of botanic nomenclature, however, since the name Streptothrix was preëmpted in 1839 for a mold belonging to the Dematiaceæ, it is not applicable to any of the organisms under consideration.

[3] Fränkel, B.: Eulenburg's Realencyklopädie der gesammten Heilkunde, 1882.

[4] Arustamoff: "Zur Morphologie und Biologie der Leptothrix," Wratsch, 1889.

von Arx[1]). The lesions are quite commonly situated in the neighborhood of the mouth and throat. Arustamoff is the only one who has succeeded in obtaining growths of the organisms observed, and it is quite uncertain whether different species are concerned in these attacks. Some investigators have supposed that a common saprophytic inhabitant of the mouth (Leptothrix buccalis) acquires pathogenic properties under certain conditions and becomes able to invade the tissues, but in view of the inadequate means of recognition for members of this group this must be regarded as altogether problematic.

Cladothrix and Nocardia Mycoses

The descriptions given in the literature do not permit a satisfactory separation of infections due respectively to the Cladothrix and the Nocardia forms, since the distinction between "true" and "false" branching has not been sufficiently regarded and recorded by all observers. Organisms related to one or the other of these groups have been found in a variety of situations: in the lachrymal canal; in a farcy-like disease of cattle (Nocard, "farcies du bœuf," probably nocardiosis); in an abscess of the brain (Eppinger,[2] who called this organism "Cladothrix asteroides," believing it to show false branching); in diseases of the lungs in various parts of the world (Japan, Aoyama and Miyamoto;[3] United States, MacCallum; South Africa, Birt and Leishman[4]); and in a number of affections designated as "pseudotuberculosis,"[5] or sometimes, but quite incorrectly, as "pseudoactinomycosis." Most, if not all, of the forms found in association with pathologic processes belong apparently to the genus Nocardia, since they are reported as showing true branching. This genus is also widely distributed in nature, and saprophytic nocardiæ (possibly with pathogenic potentialities) have been found on grasses and grains, and in soil and water. These organisms are for the most part cultivable aërobically in the ordinary culture-media at 20° C. and at 37° C. On the surface of these media (agar, gelatin, some cultures on potato) dense masses of branching filaments are formed, the growth often possessing a reddish-brown color. Espe-

[1] Von Arx: Correspondenzbl. f. Schw. Aerzte, 1889, 19, p. 161.

[2] Eppinger: Ziegler's Beiträge z. path. Anat., 1890, 9, p. 287.

[3] Aoyama and Miyamoto: Mitt. d. med. Fac. Japan, 1900, 4, p. 231.

[4] Birt and Leishman: Jour. Hyg., 1902, 2, p. 120.

[5] Flexner: Jour. Exper. Med., 1898, 3, p. 435; Schabad: Ztschr. f. Hyg., 1904, 47, p. 41; MacCallum: Centralbl. f. Bakt., Orig., 1902, 31, p. 529.

cially characteristic is the occurrence of fragmentation of the filaments, resulting in the appearance of chains of spherical bodies which are regarded as spores, and which serve to reproduce the species. These spores have not the high resistance of bacterial spores, and are more closely related to the spores of the higher fungi (see p. 547). The Nocardiæ, unlike the Actinomyces, after having been stained with fuchsin, resist decolorization with dilute acids, although not so strongly as the tubercle bacillus. Nocardiosis seems to be acquired most frequently by infection through the respiratory tract, but wound infection has been observed.

Actinomycosis

The most clearly defined and best studied affection ascribable to filamentous micro-organisms is a disease occurring chiefly in cattle, and in the horse, pig, sheep, dog, cat, elephant, and a few other animals; it is also seen occasionally in man. Although the disease was undoubtedly observed early in the nineteenth century, actinomycotic tumors being described by Leblanc[1] in 1826 under the name of osteosarcoma, it was first recognized as a specific parasitic disease by Bollinger in 1877.[2] At Bollinger's instigation the fungus was studied by the botanist Harz,[3] who gave it the name of Actinomyces on account of the ray-like structure of its growth in the tissues. Later workers have added materially to the knowledge of actinomycosis and its parasite; among these may be mentioned especially Wolff and Israel[4] and J. H. Wright.[5] Actinomycosis is essentially a suppurative process, characterized by the formation of granulation tissue and by the presence in the pus of peculiar granules, the *Drusen* of German writers. The granular masses when examined microscopically are seen to be dense rosettes of club-shaped filaments with the definite radial arrangement which has suggested the name of ray-fungus.

Characteristics of Actinomyces.—The features of this interesting parasite may be considered as presented (1) in the tissues, and (2) in cultures.

[1] Leblanc: Jour. de méd. vétérin., 1826, p. 333.

[2] Bollinger: Deut. Ztschr. f. Tiermed., 1877, 3, p. 334.

[3] Harz: Deut. Ztschr. f. Tiermed., Suppl., 1878, 4, p. 125.

[4] Wolff and Israel: Virchow's Arch. f. path. Anat., 1891, 126, p. 11.

[5] Wright, J. H.: Pub. Mass. Gen. Hosp., Boston, 1905; Jour. Med. Res., 1904–5, 8, p. 349.

(1) As found in the animal tissues, the largest of the colonies of the ray-fungus are visible to the naked eye as minute yellowish granules, the individual rosettes of which the granules are composed averaging perhaps 30 to 40 μ in diameter, though they may sometimes reach a much larger size (200 μ). A granule may consist of a single rosette, or, as in the case of the largest granules, may be compacted of several. Three kinds of structures make up a typical Actinomyces rosette: (*a*) a central core of branching filaments irregularly disposed, but with a general radial arrangement; (*b*) at the periphery refringent, club-shaped bodies; (*c*) spherical, coccus-like bodies. The colonies may be examined in fresh unstained preparations in which the clubs may be plainly seen, or some stain like eosin may be used, which colors the sheath of the clubs. The filaments stain by Gram's method, which is consequently well adapted for treating sections of the affected tissues.

Fig. 122.—Colonies of Actinomyces on agar and blood-serum (Wright).

(*a*) The filaments exhibit true branching, are often curved, sometimes spirally, and are thickly interlaced in a network like the mycelium of the higher fungi. The individual threads are, for the most part, slender (about 0.5 μ in diameter), and are composed of granular protoplasm surrounded by a delicate sheath. In the older colonies fragmentation or segmentation of the cell-substance may be observed, giving the appearance of chains of cocci.

(*b*) The club-shaped bodies at the margin of the Actinomyces granule are conspicuous for their high refringency and general structureless, homogeneous appearance. They are pear-shaped swellings of the terminal ends of the filaments, and arise as distinct transformations of the latter. In young colonies the hyaline substance of which the clubs are composed is soft and may be dissolved in water, but as the age of the colony increases, the clubs become of firmer consistency. The clubs are found especially in colonies from lesions where there is evidence that the tissue is displaying

resistance to the inroads of the micro-organism; when resistance to invasion is slight, they are absent, filaments alone being found. Clubs are, as a rule, more common in bovine than in human lesions.

(*c*) Coccus-like bodies have been reported by a number of observers as being present in the Actinomyces granules. This designation has doubtless been applied to bodies of diverse nature. The segmentation of a filament into a chain of spherical structures has been already mentioned; these bodies are then usually described as "cocci." Some of the "coccoid" bodies are perhaps real micrococci, secondary invaders of the suppurating actinomycotic lesion, and some are perhaps simply the ends of clubs that first appear in focusing a lens down upon a rosette.

Fig. 123.—Smear preparations of Actinomyces from broth culture. × 1500 (Wright).

(2) In cultures the essential features of the Actinomyces rosette have been reproduced. The smaller colonies are rounded masses of branching and interlacing filaments. As the filaments become older they tend to break up into segments, and the largest colonies are dense, opaque masses of short filaments and rod forms. Clubs are not formed in ordinary broth (Fig. 123), but only in the presence of blood, blood-serum, or serous pleuritic fluids (Wright), where, however, their development is inconstant and dependent to some extent on unknown factors. Sections of the colonies stain well by Gram's method; according to Wright, the clubs are best shown in paraffin sections stained according to Mallory's method (Fig. 124).

Much discussion has centered around the biologic significance of the different elements of the Actinomyces colony. The clubs were at one time supposed to be reproductive organs of some sort, but little or no evidence has been adduced in support of this view, which is now practically abandoned. As already pointed out, the

clubs are formed only when the colony is in contact with animal fluids that oppose a certain degree of resistance to the growth of the organism. They are consequently thought by many to be degeneration products due to the unfavorable conditions encountered by the tips of the filaments, that is to say, to lack of space or to the restraining influence of the body-cells or body-fluids; they have also been looked upon as protective devices for resisting the destructive action of the body-fluids.

The bodies described as "coccus forms" are in some cases foreign micro-organisms present in the tissues together with the Actinomyces and constituting a mixed infection; in others, and perhaps the majority of cases, they are the products of the degeneration of a filament. The view that any of the coccus-like bodies are spores or are in any wise related to the normal reproduction of the species has little in its favor and is not countenanced by any feature in the life-history of the organism in culture.

Fig. 124.—Colony of Actinomyces with well-developed "clubs" at the periphery in a nodule in the peritoneal cavity of a guinea-pig inoculated with a culture from another guinea-pig. Paraffin section. Low magnification (Wright). (Photograph by Mr. L. S. Brown.)

The Cultivation of Actinomyces.—Much perplexity has been caused in the study of this organism—(1) by the error of mistaking Nocardia forms for Actinomyces, and (2) by the failure to recognize the occurrence of mixed infections. A large part of the difficulty of separation has arisen from the fact that Actinomyces is at first essentially an anaërobe of slow growth, and is, therefore, not easily freed from the contaminating microbes that are often present in the pus of actinomycotic lesions. Wright recommends the following procedure for the isolation of Actinomyces: The granules, prefer-

ably from a closed lesion, are first thoroughly washed in sterile water or broth and then crushed and disintegrated between two sterile glass slides. Bovine material may well be examined microscopically to see if a goodly number of filamentous masses are present, for in some of the granules degeneration has gone so far that no growth can be expected. When filaments are present, the crushed fragments of the granule are transferred with the platinum loop to fluid 1 per cent. dextrose agar (at 40° C.) contained in test-tubes filled to a depth of 7 or 8 centimeters. The material is thoroughly distributed throughout the melted agar and the tube placed in the incubator. Several tubes should be prepared. If the number of filaments introduced is considerable, characteristic colonies of Actinomyces develop in the depth of the agar, especially in a shallow zone about 5 to 12 mm. below the surface. If many colonies of contaminating organisms appear in the tube, it is probably not worth while to continue the attempt at isolation. To guard against possible loss of material from an important case, it is desirable at the outset to place a number of washed granules on the sides of the sterile tubes plugged with cotton and left at room temperature in the dark. Preserved thus for two or three weeks, the contaminating bacteria perish by drying, and in case the original agar tube cultures have proved unsuccessful, the dried granules may be treated in the same manner as were the fresh ones.

When the presence of the characteristic colonies and the absence of a large number of contaminating colonies have been determined, cut out pieces of agar containing colonies by a stiff platinum wire with a flattened oval bent end. With a low power of the microscope select an isolated colony for transplanting, carefully cut out the small piece of agar containing it, the diameter of the piece not to exceed 2 mm., and transfer the piece with a platinum loop to a tube of sterile broth, where it should be thoroughly shaken up to free it from any adherent bacteria. If there is reason to believe that the small piece of agar has been very much contaminated with bacteria, wash it in a second tube of broth, then transfer with a platinum loop to a tube of melted glucose agar (at 40° C.). Immerse the transplanted piece deep in the agar and place the tube in the incubator. If the colony is capable of growth, and contamination has been avoided, a good-sized colony in pure culture will result, and from this transfers to various culture-media may be made.

The most consistent descriptions of the cultural characters of Actinomyces are found in the work of Wolff and Israel, and in the later monograph of Wright. Discrepancies with the statements of other writers, notably with those of Bostroem, are, according to Wright, most reasonably explained by assuming that the latter worked with impure cultures. Actinomyces grows well in 1 per cent. dextrose agar. In this medium at 37° C. the colonies in the depth—that is, some 10 mm. below the surface—appear as irregularly shaped, opaque, whitish nodules which may reach a diameter of 2 to 3 mm. in a week (Fig. 125). Surface growth upon ordinary nutrient agar, upon dextrose or glycerol-agar, or upon blood-serum, is never luxuriant, and sometimes fails to appear altogether. Stab cultures in dextrose agar give a dense grayish streak of small colonies along the lower part of the line of inoculation, no growth occurring immediately below the surface. In broth a good growth usually occurs in the form of solid, whitish, mulberry-like granules at the bottom of the tube; there is never growth at the surface. After being under cultivation for some time most strains form flaky, friable, amorphous masses. The broth does not, as a rule, become cloudy. The predilection of this organism for anaërobic growth is shown by these peculiarities.

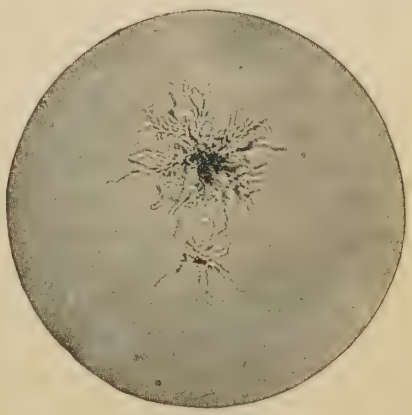


Fig. 125.—Small colonies of Actinomyces bovis in the depths of an agar culture (Wright).

Upon potato, growth is scanty and not distinctive. Milk is not a favorable medium (Wright). A temperature of about 37° C. seems necessary for successful cultivation of the true Actinomyces. At the ordinary room temperature, when any growth at all takes place, it is very slight. Considerable resistance is shown to drying, most strains being alive after having been dried for fifty days on the walls of the test-tube; some observers have reported continued vitality in cultures dried for upward of a year.

The question whether there is more than one species of Actinomyces has been considerably obscured by the confounding of Nocardia (and Cladothrix?) with this organism, as already stated. Apart from the illegitimate confusion of names thus resulting, some authors have held to a plurality of species, and have, for example, designated organisms from human and bovine sources respectively as Actinomyces hominis and A. bovis. The most recent and thoroughgoing studies, however, indicate that there is no substantial difference in the cultures from man and from cattle, and there is at present no evidence that more than one species is involved in the causation of actinomycosis.

Pathogenicity for Cattle and Other Animals.—The location of actinomycotic lesions in cattle is eminently characteristic. The majority are found in or about the head; the lower jaw and the tongue in particular are affected with great frequency; hence the common names of "lumpy jaw" and "wooden tongue" for this disease. In addition to the growth in the tongue and maxillary bones, actinomycotic lesions occur in the pharynx, in the lungs, in the skin, lymph-glands, and subcutaneous tissue, especially of the head and neck, and occasionally in other organs, notably the udder and liver. The growth of the parasite leads in most situations to the formation of a hard tumor which gradually increases in size and burrows into the adjacent tissues, softening and disintegrating the bony structure of the head, and at the same time forming new tissue, so that great distortion often ensues. The extension of the disease takes place by gradual invasion of the contiguous tissues, metastases being uncommon. When death occurs it is not, as a rule, due to any toxic effect, but wholly to the mechanical action of the tumor in pressing upon or occluding the respiratory passages or in interfering with the taking or mastication of food.

In the suppurating mass of the tumor are found the characteristic granules or "glands" (*Drusen*), composed of rosettes, as already described; these structures are so typical that their discovery by microscopic examination in a case of obscure suppuration is sufficient to establish a diagnosis. The new growth—granulation tissue—consists chiefly of epithelioid and spindle-shaped connective-tissue cells; small giant-cells are also sometimes present. To the naked eye, actinomycotic lesions in the lung and udder often resemble tuberculosis nodules, and have undoubtedly at times been

mistaken for the latter; but microscopic examination leaves little ground for confusion.

Generalized actinomycosis is rare. When it occurs, the blood-stream rather than the lymph seems to be the channel by which the disease is spread. Secondary abscesses are found mainly in the liver. The horse and the pig are not often affected, the sheep still more seldom. The lesions in these animals are substantially of the same character as those in cattle.

Experimental inoculations of cattle, swine, dogs, rabbits, guinea-pigs, and other animals have given a large proportion of absolutely negative results. In some cases investigators (*e. g.*, Wolff and Israel) have obtained the formation of tumors containing Actinomyces colonies with typical "clubs," but in general definite positive results from inoculation are few in number. Wright inoculated eighty-six animals with pure cultures and obtained lesions referable to the micro-organism in but thirty; the lesions were, moreover, insignificant in most cases. A rapid diminution in the virulence of the culture appears to take place under cultivation. Individual susceptibility doubtless plays a large part in determining the course of infection.

Occurrence of Actinomycosis in Man.—This disease is observed in man from time to time, although it is not common. Sanford[1] has obtained data concerning about 700 cases in the United States up to 1923. The disease seems especially prevalent in the upper Mississippi valley. Actinomycotic affections of the bone are relatively infrequent, the disease being confined to the softer parts in most cases.

The tissue changes produced by the presence of the parasite in man are also somewhat different from those in cattle. There are generally a slighter production of new tissue and a more extensive softening and suppuration, which gradually spread to adjacent parts. Generalization of the infection is more common in man than in cattle. The disease may terminate fatally in a few weeks through secondary infection or formation of emboli, or may drag along in a chronic form for many years. Spontaneous healing has been observed. Compounds of iodin (potassium iodid) have, for unknown reasons, high therapeutic value in actinomycotic affections both in man and in cattle.

[1] Sanford: Jour. Amer. Med. Assoc., 1923, 81, p. 655.

Method of Infection.—The parasite of actinomycosis usually enters the body of cattle from the mouth, pharynx, or other point in the upper alimentary tract—at least, this is inferred from the peculiar and frequent localization of the disease in the jaw and head, especially since in cattle metastases are rare. There is reason for incriminating the tonsils and carious teeth as starting-points for actinomycotic lesions in man. Many patients give a history of chewing straws or grain. Although the usual point of invasion thus seems to be quite definitely determined, much uncertainty prevails concerning the immediate source of the invading parasite.

Transmission by direct contagion from one animal to another or from cattle to man has never been satisfactorily established. Many of the cases of actinomycosis reported in man are among persons who have not been engaged in agricultural pursuits and, so far as discovered, have not come in contact with any preëxisting case in animals or man.

A number of observers have encountered fragments of grain (barley or corn) embedded in the soft tissues of the mouth and forming the apparent core of actinomycotic growth. On the basis of such findings the view has been advanced that the natural habitat of the Actinomyces fungus is on grain (especially barley—Bostroem[1]), and that it is introduced into the tissues either through slight scratches or wounds of the mucous membranes of the mouth or throat, or through direct penetration into the tissues of an infected awn or other sharp particle. In support of this conception it is pointed out that the parasite has been reported as commonly found on grain (Johne, Bostroem). Indirectly confirmatory, too, is supposed to be the fact that in a number of cases of actinomycosis in man there is a suggestive history, such as the habit of chewing grain, nibbling at grain stalks, and the like. It must be said, however, that the evidence that the thread-like fungus found on barley grains is identical with the true Actinomyces is quite inadequate, and the wide distribution in nature of the latter parasite remains to be demonstrated. As regards the presence of foreign bodies in actinomycotic lesions, it is not necessary to assume that such a body is the special vehicle of the germ. It may reasonably be maintained that Actinomyces is normally present in the mouth, and that its invasion of the tissues is simply facilitated by the irritation caused

[1] Bostroem: Ziegler's Beiträge z. path. Anat., 1890, 9, p. 1.

by the foreign particle. The latter opinion is held by at least one high authority upon actinomycosis (Wright).

Mycetoma or Madura Foot

This disease, as the name implies, usually affects the foot; occasionally the hand is attacked, rarely other parts of the body. It is primarily a disease of warm climates. The part involved shows at first a small swelling, which slowly enlarges and softens, discharging a viscid, slightly purulent fluid in which are minute granular particles. The foot, when affected, becomes greatly enlarged and misshapen (Fig. 126). As in actinomycosis, the bones are often involved. Extension of the infection by the formation of secondary abscesses is said not to occur, and the internal organs are never affected. Three varieties of the malady have been distinguished according to the color of the granules in the diseased tissue: (1) white or yellowish granules, the most common type; (2) a less common black; and (3) a rare red variety. The color of the granules is the only distinguishing mark; the other features are said to be practically identical.

Fig. 126.—Madura foot—mycetoma (Musgrave and Clegg).

White Variety.—It seems probable that some of the cases in which pale yellowish or ochroid granules have been reported were infections with Actinomyces. Some observers believe that all cases of the white type are in reality actinomycotic. The granules in this type are composed of an agglomeration of colonies which, like Actinomyces, are made up of a network of filaments. These filaments, moreover, terminate at the margin of the colony in the club-shaped swellings so characteristic of the Actinomyces fungus (Fig. 127). The cultural features of the organism isolated from the yellow granules in mycetoma do not, however, correspond, as reported (Vincent), with those of Actinomyces bovis, and it must be regarded as possible that the apparent discrepancies are due to the occurrence of mixed infections, which have elsewhere proved so fruitful a source of error in the study of these organisms.

Musgrave and Clegg[1] have successfully carried out inoculation experiments upon monkeys with a pure culture of a fungus found in a case of a white variety of mycetoma. The parasite is distinct from Actinomyces and differs in some respects from the organism described by Wright. A very full bibliography of mycetoma is given by these writers.

Black Variety.—The black granule (melanoid) variety of mycetoma, although considered clinically and anatomically like the more common form in which whitish or yellowish granules are found, has associated with it a fungus very different from Actinomyces. The parasite of the dark granules has been studied especially by Wright.[2] It is a large fungus with thick branching filaments and transverse septa like the hyphæ of the higher molds. The protoplasm of the hyphæ is impregnated with black pigment. Wright obtained ready growth on the ordinary culture-media under aërobic conditions. Spore formation was not observed. This organism must apparently be ranked with the true Hyphomycetes rather than with the Trichomycetes, although little is yet known of its life-history.

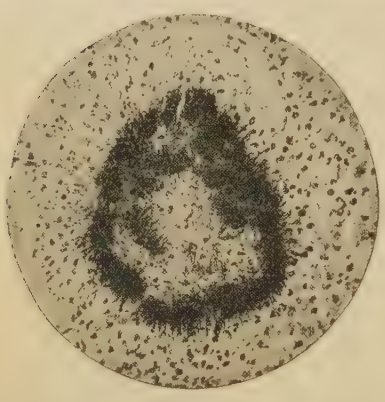


Fig. 127. –Mycetoma. Section of tumor of inoculated dog. × 390 (Musgrave and Clegg).

Erysipeloid

An erysipelas-like eruption, described under the name erysipeloid, has been noted by clinical writers in various parts of the world.[3] In some instances there seems to be a direct epidemiological and bacteriological relationship to swine erysipelas, an infection of swine caused by an organism (Erysipelothrix) placed by taxonomists close to the Actinomyces; in other instances a specific but related microbe is thought to be concerned. The bacillus of mouse septicemia belongs in this group.

[1] Musgrave and Clegg: Philippine Jour. Sci., B, 1907, 2, p. 477.

[2] Wright: Jour. Exper. Med., 1898, 3, p. 421.

[3] Cf. Klauder: Jour. Amer. Med. Assoc., 1926, 86, p. 536.

CHAPTER XXIX

THE BLASTOMYCETES

The organisms known as blastomycetes, saccharomycetes, or yeasts are fungi characterized especially by the mode of multiplication or cell-division called *budding* (*Blastomycetes* = budding fungi) (Fig. 128). The cells are spheroid or egg-shaped in form, and pos-

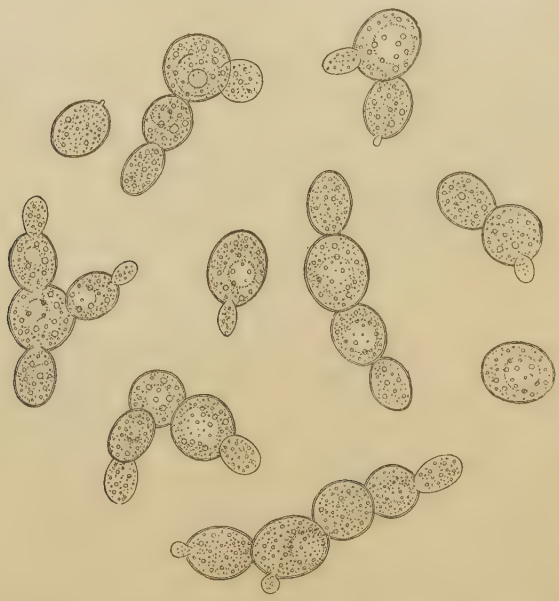


Fig. 128.—Yeast cells. Brewer's (top) yeast actively vegetating. The large internal vacuoles and the small fat-drops are shown, as are also buds, in various stages of development, and the cell-wall. Nuclei not visible. (Highly magnified) (Sedgwick and Wilson).

sess a well-defined cell-wall of cellulose. As a rule, they are much larger than the bacteria. Many yeasts, or, according to some authorities, all the "true" yeasts, form spores under suitable conditions by endogenous cell-division; the usual number of spores (ascospores) is four (Fig. 129), but some yeast-like organisms have been found that form eight spores (Schizosaccharomyces octosporus, Beijerinck).

Under certain conditions some of the higher molds develop yeast-like cells which multiply by budding and show many of the characteristics of the common yeasts. The relationship of the yeasts to other fungi has not been clearly made out, and some botanists would deny these organisms any standing as an independent group, regarding them simply as a stage or phase in the life-history of higher organisms. Different names are given to various yeast-like organisms, but without any absolute precision or uniformity. The term *torula* is often applied to certain wild or uncultivated yeasts, usually spherical in shape, non-spore-producing, and with relatively slight fermentive power. *Oïdium* is a name given to some forms that show a marked tendency to grow out into long threads or hyphæ.

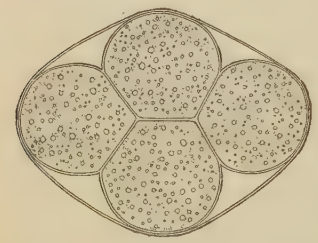


Fig. 129.—Spores of yeast (ascospores). Four spores in a cell of brewer's yeast (Saccharomyces cerevisiæ) (Sedgwick and Wilson).

Yeasts have long been known for their ability to produce alcoholic fermentation, and the technical study of these organisms has been chiefly carried on in connection with brewing and other practical occupations. A variety of familiar processes, such as the rising of dough, are effected through their agency. It is not possible, in the compass of this work, to do justice to the relations of yeast to the various fermentation industries.[1]

The action of the enzymes produced by yeasts has been the object of particular interest. Biologists have known for a long time that the cultivated species of yeasts elaborate at least two enzymes, namely, invertase, which has the power of changing or inverting saccharose into dextrose and levulose ($C_{12}H_{22}O_{11}+H_2O=C_6H_{12}O_6+C_6H_{12}O_6$), and maltase, which splits a molecule of maltose into two of dextrose; but for many years it was disputed whether the further conversion of dextrose into alcohol and carbon dioxid was due to a specific enzyme. Buchner[2] in 1897 first showed

[1] The reader is referred to the following books, dealing with alcoholic fermentation: Lafar: "Handbuch der technischen Mykologie," vols. 4 and 5, Jena, 1905–06–07; Jörgensen: "Micro-organisms and Fermentation," trans., London, 1900; Hansen: "Practical Studies in Fermentation," London, 1896; Oppenheimer: "Die Fermente und ihre Wirkungen," Leipzig, 1900.

[2] Buchner: Chem. Ber., 1897, 30, pp. 117, 1110, 2668.

that this action also was caused by an enzyme, which, however, unlike invertase and maltase, was closely bound to the cell protoplasm and did not diffuse out into the surrounding medium during the life of the cell. A solution of the alcoholic enzyme or zymase is obtained by rupturing the living yeast-cell, by the method of grinding pressed yeast with sand, and then subjecting the moistened mass to high pressure. The filtered liquid that is expressed from the yeast cells is able to induce the alcoholic fermentation of a sugar solution. The objection that the fermentation was due to particles of living yeast plasma contained in the yeast juice was met by centrifugalizing the latter, when it was found that the liquid portion possessed the same power of fermentation as that containing the plasma.

The use of pure cultures of yeasts placed the brewing industry for the first time on a scientific basis. In earlier days the beer wort was commonly invaded by bacteria and "wild" yeasts from countless sources, and the quality of the product was hence uncertain and frequently unsatisfactory. Although Pasteur recognized the share of bacteria in causing "diseases" of beer and wine, he did not fully appreciate the part played by wild yeasts, and it was the service of Hansen, the Danish bacteriologist, to trace much of the common deterioration of beer to the latter source. Hansen showed, further, that it was possible to avoid the interference of wild yeasts by inoculating the wort with cultures of suitable yeasts produced from a single cell under conditions that precluded outside contamination. The method of pure yeast cultures devised by him has been universally adopted, and special forms of apparatus for the development of pure yeast are in general use in all large breweries.[1]

Pathogenic Yeasts.—The study of the pathogenic activities of blastomycetes in human beings dates, practically, from the discovery by Busse in 1894 of a generalized fatal infection apparently caused by a yeast.[2] In this case, besides the chief lesion in the tibia, all accessible lymphatic glands were found to be enlarged; the patient died thirteen months after the appearance of the tibial abscess, and the yeast was found in similar abscesses in the ulna and one rib, and also in the lung, left kidney, and spleen. A somewhat similar organ-

[1] See, for an excellent description of modern brewing, the "American Handy Book of Brewing, Malting, and Auxiliary Trades," Wahl and Henius, Chicago, 1902.

[2] Busse: Centralbl. f. Bakt., 1894, 16, p. 175.

ism was described by Curtis[1] as the apparent cause of myxomatous tumors. Gilchrist[2] was the first to observe and describe a well-defined skin disease, now usually termed blastomycetic dermatitis or oïdiomycosis, which is caused by a yeast-like organism. Since that time a number of cases of this characteristic skin disease have been reported, especially from the vicinity of Chicago, and the infection has been made the subject of a comprehensive monograph

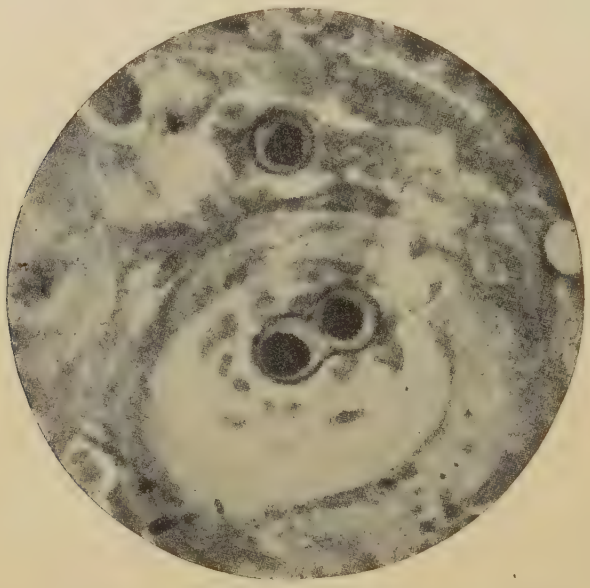


Fig. 130.—Blastomyces. A pair of organisms in an incipient intraepithelial abscess, also a single mature form between epithelial cells. × about 1200 (Ricketts).

by Ricketts.[3] The organisms occur in the tissues only in the budding, yeast-like stage. The larger cells (Fig. 130), which are from 10 to 17 μ in diameter, are spherical and possess a homogeneous, double-contoured capsule, the cytoplasm being sometimes finely, sometimes coarsely, granular and often vacuolated.

Considerable difficulty is experienced in obtaining cultures directly from fresh tissue, but after growth once appears cultures can be readily maintained on all the usual laboratory media. Freshly isolated cultures grow slightly better on Löffler's blood-serum.

[1] Curtis: Ann. de l'Inst. Pasteur, 1896, 10, p. 449.
[2] Gilchrist: Johns Hopkins Hosp. Rept., 1896, 1, p. 269.
[3] Ricketts: Jour. Med. Res., 1902, 6, p. 377.

On agar after three to seven days small white colonies develop which show under a low power numerous aërial hyphæ. The growth is often distinctly mold-like, especially in dry cultures. The substratum becomes penetrated with a mass of mycelial threads and gradually turns to a brown color. Upon glucose-agar there is a somewhat more profuse growth. In broth a fluffy, coherent, globular growth occurs at a fixed point in the fluid, the supernatant fluid remaining clear. Gelatin is not liquefied. Milk turns slightly alkaline and shows some digestion of casein without coagulation.

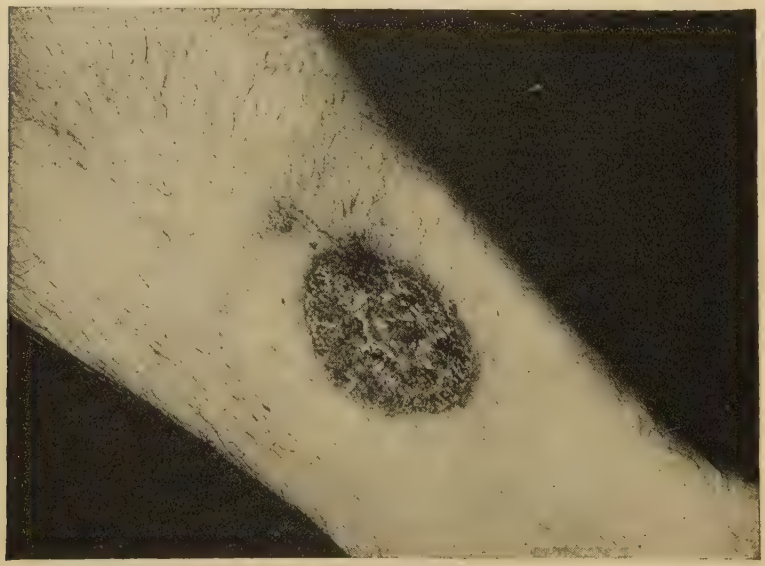


Fig. 131.—Blastomycosis. Old partially healed ulcer of the leg (Irons and Graham).

On potato the growth is rather rapid, and in some cultures a large number of budding forms are found with few hyphæ. No gas is produced in dextrose solutions. Guinea-pigs and rabbits inoculated subcutaneously develop abscesses containing yellowish, cheesy material in which the blastomycetes are present in the budding form. Generalized infection sometimes results. In man, blastomycetic dermatitis follows a substantially uniform course. There is first noticed a small papule, most commonly on the hand, forearm, or face, which yields a viscid pus and gradually enlarges its area (Fig. 131). In a great majority of cases there is absence of lymphatic glandular involvement. The disease may extend over ten or

twelve years and cause great cicatricial deformity. Potassium iodid has been used in some cases with distinctly beneficial results.

A number of cases of generalized blastomycosis terminating fatally have been reported. In the majority of these the lung seems to have been the seat of primary infection. Multiple superficial and deep abscesses occur, and both clinical and pathologic findings are rather characteristic.[1]

A singular and more fatal disease of a similar character has been observed in the San Joaquin Valley on the Pacific slope of the United States.[2] Although the organism found was at first thought to be a protozoön, and the disease is still termed "coccidioidal granuloma," there is now no doubt that the parasite concerned is a blastomyces or "oïdium." Generalized infections are the rule and are almost invariably fatal. Cutaneous lesions are often entirely secondary and may even be wholly absent.[3]

The specific micro-organisms from the Pacific coast, when compared with the blastomycetes from the Chicago cases, show some differences in behavior on culture-media, but the distinction upon which most stress falls is that the Pacific coast fungus occurs in the tissues only in the mycelial form, never in the budding form. In pus and in culture-media budding forms very similar to those of "blastomycetic dermatitis" have been observed. The organism of coccidioidal granuloma is also characterized by endogenous spore formation, and proliferation in the tissues appears to be by sporulation.

A number of writers, chiefly Italian observers (*e. g.* Sanfelice[4]), have sought to establish some relation between blastomycetes and malignant tumors, but the evidence advanced in favor of such a causal connection is totally inadequate. The resemblance between yeasts and the cell inclusions in cancerous tissue seems to be purely superficial.

[1] Irons and Graham: Jour. Infect. Dis., 1906, 3, p. 666.
[2] Ophüls: Jour. Exper. Med., 1901, 6, p. 443.
[3] Brown: Jour. Amer. Med. Assoc., 1907, 48, p. 743.
[4] Sanfelice: Centralbl. f. Bakt., 1902, I, Orig., 31, p. 254.

CHAPTER XXX

THE HYPHOMYCETES

The organisms known as *Hyphomycetes* or *Eumycetes* are sometimes grouped with the bacteria under the general head of fungi. They are, however, quite distinct from the bacteria, and are more closely related to the higher algæ; perhaps they should be regarded phylogenetically as forms that have lost the chlorophyl they once possessed as the result of taking up a saprophytic or parasitic mode of life.

The filamentous character of the growth is one of the features of this group of organisms. Each filament is termed a *hypha*, and the whole matted, felt-like mass of interlacing filaments is called the *mycelium*. In the lower Hyphomycetes—those showing the closest resemblance to the algæ—each filament is a single, simple, or greatly branched multinuclear cell. In the higher forms each filament consists of a row of cells set end to end. The lower Hyphomycetes, or Phycomycetes, are further distinguished by their mode of reproduction, which is both asexual and sexual, while the higher fungi probably lack either wholly or in part the sexual mode of development. The common white cottony mold (Mucor mucedo), which grows on damp bread, horse-dung, etc., is a familiar example of the Phycomycetes. In this species the asexual form of reproduction is the more common. From the single-celled, finely branched mycelium rise erect, unbranched hyphæ, near the apex of each of which a septum forms. The tip of the hypha then swells into a globular *sporangium*, within which numerous oval spores develop; the wall of the ripe sporangium ruptures easily and the spores are discharged by the swelling of the gelatinous mass in which they are embedded (Fig. 132). Under certain conditions conjugation of two cells precedes spore-formation (sexual reproduction). Lateral, club-shaped outgrowths occur in neighboring hyphæ and constitute the so-called gametophores. When the tips of two gametophores come in contact, they fuse, transverse septa are formed, and a *zygospore* is the result. From the matured

zygospore a germ-tube arises and may at once develop a sporangium at the apex (Fig. 133).

The higher Hyphomycetes reproduce usually, perhaps exclusively, by asexual spore-formation. In one of the principal groups—Ascomycetes—spores are formed within the hyphæ in asci or tubular spore-cases. In some cases the fertile hyphæ are inclosed within an envelop called the perithecium, which is composed of closely interwoven sterile hyphæ. The very common blue-green mold, Penicillium, frequently found as an air-contamination on gelatin

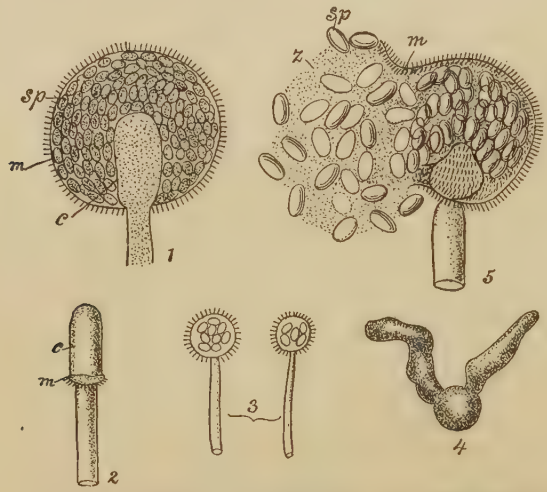


Fig. 132.—Mucor mucedo: 1, A sporangium in optical longitudinal section: *c*, columella; *m*, wall of sporangium; *sp*, spores; 2, a ruptured sporangium with only the columella (*c*) and a small portion of the wall (*m*) remaining; 3, two smaller sporangia with only a few spores and no columella; 4, germinating spores; 5, ruptured sporangium of Mucor mucilaginus with deliquescing wall (*m*) and swollen interstitial substance (*z*); *sp*, spores (after Brefeld).

or agar plates, multiplies by the direct formation of gonidia or spores from the segmentation of portions of the hyphæ; the terminal portion of certain filaments breaks up into finger-like branches, and these branches become constricted into rows of oval spores (Fig. 134). It is still uncertain how far the reproduction of certain of the molds is dependent upon external conditions and how far upon internal relations. Possibly in some cases cell-conjugation precedes the formation of reproductive bodies. There is no consensus concerning the classification and inter-relationship of these organisms, and in many cases the life-history is incompletely known.

The molds prefer an acid to an alkaline reaction, and are frequently a source of trouble to the housekeeper from their tendency to attack fruit preserves and similar substances. The ability of the hyphæ to force their way through narrow spaces enables these organisms to invade the contents of any receptacle not tightly sealed. Culture-media in bacteriologic laboratories are often contaminated by molds if the cotton plugs are allowed to become moistened, the mold hyphæ readily making their way between the cotton filaments. The spores of molds are well-nigh ubiquitous, and are usually more abundant than bacteria in ordinary air.

Fig. 133.

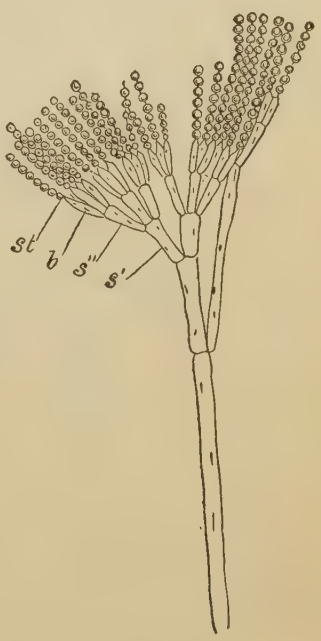


Fig. 134.

Fig. 133.—Mucor mucedo. Different stages in the formation and germination of the zygospore: 1, Two conjugating branches in contact; 2, septation of the conjugating cells (*a*) from the suspensors (*b*); 3, more advanced stage in the development of the conjugating cells (*a*); 4, ripe zygospore (*b*) between the suspensors (*a*); 5, germinating zygospore with a germ-tube bearing a sporangium (after Brefeld).

Fig. 134.—Penicillium crustaceum. Conidiophore with verticillate branches, *s'*, *s''*, *b*, *st*, sterigmata abstricting chains of conidia; × 540 (Strasburger).

Certain species of Aspergillus are commonly used in Asiatic countries for making fermented drinks. The conversion of the starch of the rice grains into sugar by one of these molds is the first step in the production of sake and other alcoholic liquors. The molds of this genus seem particularly rich in enzymes, and their

products have been utilized to some extent in commercial preparations such as "taka diastase."[1]

Many of the molds are able to attack the tissue of the higher plants and to cause widespread and serious plant diseases. The potato-rot, caused by the fungus Phytophthora infestans, is a familiar example. The infection of rye and other grains with the fungus Claviceps purpurea may entail disastrous consequences for man, since the use of infected grain has been found to cause the condition of poisoning known as ergotism. A poisonous substance called ergot has been obtained from the moldy grain, and its physiologic qualities have caused it to be used in obstetric practice for producing contraction of the uterus. Other grains and vegetable foods are not infrequently attacked by fungi which generate toxic compounds, so that the use of moldy foods by man or by the domestic animals is attended with some danger. In certain cases the fungi producing the poisonous substances have been carefully studied, as in the case of ergot; in others, little is known about them.

While a great many fungi are parasitic upon the higher plants, a relatively much smaller number are known to possess pathogenic properties for the higher animals, including man. Plaut[2] has divided the fungi pathogenic for man into three groups, according to their pathogenic effects: (1) The molds in the narrower sense (Mucor and Aspergillus); (2) the fungus of thrush; (3) the fungi infecting the skin.

1. Certain widespread and common molds are sometimes found associated with pathologic conditions. In a fatal infection in man described by Paltauf[3] a representative of the genus Mucor was found in abscesses in the lungs, peritoneal cavity, and brain. Instances of infection with several species of molds of the common genus Aspergillus are fairly numerous. Lung infections and ulcerations of the ear are fairly common. Most of the reported cases of lung infection are among bird-fanciers, especially those having to do with the care of pigeons. These birds are liable to a pulmonary form of aspergillosis, and the evidence is fairly convincing that transmission of the disease from pigeons to man can take place.

[1] For a comprehensive monograph see Thom and Church: "The Aspergilli," Baltimore, 1926, pp. 272.

[2] Plaut: Kolle and Wassermann's Handbuch, 2nd ed., 1, p. 549.

[3] Paltauf: Archiv f. Path. Anat., 1885, 102, p. 543.

Experimental infection has been produced in birds by inhalation of spores. Birds and mammals can also be fatally infected by intravenous inoculation with Aspergillus spores (Fig. 135).

2. An infection of the mucous membrane, usually of the mouth of infants, rarely seen in adults, is known as thrush (Ger., *Soor*). The fungous growth is usually localized in the form of white patches on the mucous surface attacked, but generalized infection also may occur, though it is not common. Birds and the lower animals are occasionally attacked spontaneously and may also be artificially infected. A slight degree of immunity may be obtained by repeated inoculation of non-fatal doses of the living parasite, but not with its soluble products. Two morphologically distinct varieties of fungi have been found in thrush. One of these, the large-spored, gelatin-liquefying variety, is much more common than the other, and practically all of the recorded observations and experiments relate to this form. The spores or yeast-like bodies found in the large variety are about 5.6 μ long and 4 μ in diameter, while in the small variety they are usually spherical and are from 1.9 to 3.8 μ in diameter. The nomenclature of the thrush fungus is in almost hopeless confusion. Plaut[1] prefers Monilia candida, though probably the name in most common use is Oïdium albicans.

Fig. 135.—Aspergillus fumigatus, from the lung of a parrot (Plaut).

3. The dermatomycoses or skin diseases caused by fungi form a group of respectable dimensions. There is much uncertainty and difference of opinion concerning the nature and systematic position of the various parasites, and a full consideration of the points in dispute is here impossible. In favus, a not uncommon disease of the scalp and other parts of the body, characterized by a dry, yellowish, honeycomb-like incrustation, some investigators would recognize as many as nine different varieties of fungi at times responsible for this condition, while others regard the infection as

[1] Plaut: Kolle and Wassermann's Handbuch, 2nd ed., 1, p. 535.

due to a single highly pleomorphic fungus. The trend of opinion seems toward the latter view. Wälsch[1] has shown that the parasite of favus in mice can be adapted to growth in the human skin, where it becomes converted into the characteristic fungus of human favus.

The skin affection usually known as ringworm (Herpes tonsurans) is caused by two, possibly more, varieties of the genus Trichophyton. A large-spore and a small-spore variety of the fungus are generally recognized, the latter being the more common. The infection is communicated from man to man, and may also be contracted from the horse, cat, dog, and other domestic animals. The fungi of ringworm and favus are able to penetrate the underlying layers of the skin and to cause pathologic changes in the tissue elements. In this respect they are more highly or more definitely parasitic than certain other fungi which vegetate on the superficial layers of the skin and do not produce marked pathologic lesions. Pityriasis, or Tinea versicolor, a dry, scaly skin eruption, is caused by one of these semi-saprophytic fungi, Microsporon furfur.

Sporothrix.—A peculiar ulcerative infection observed in the hand and arm of a patient presenting himself at the surgical clinic of the Johns Hopkins Hospital in 1896 was shown by Schenck[2] to be due to a fungus usually classed in the genus Sporothrix. Many other cases of sporotrichosis have since been observed in man and in such domestic animals as the dog and horse.[3] There is often a history of some minor injury to the hand or foot followed by the development of a chain of nodules along the line of the lymphatics. In some cases, however, especially those reported in France, the infection is accompanied by multiple wide-spreading abscesses in various parts of the body.

The fungus shows mycelial threads and conidia when cultivated in broth and agar. Some, like Schenck's original culture, liquefy gelatin; others lack the power of liquefaction. An abundant growth occurs on potato, and is often, but not invariably, accompanied with considerable pigmentation. Inoculation of the dog and mouse proves the pathogenic power of the organism; the

[1] Wälsch: Prag. med. Wchnschr., 1898, 23, pp. 206, 219.

[2] Schenck: Bull. Johns Hopkins Hosp., 1898, 9, p. 286.

[3] Hektoen and Perkins: Jour. Exper. Med., 1900–01, 5, p. 77; Page, Frothingham and Paige: Jour. Med. Res., 1910, 23, p. 137; Walker and Ritchie: Brit. Med. Jour., 1911, 2, p. 1.

guinea-pig is relatively insusceptible. In the tissues the conidia seem to multiply by budding, and true mycelial formation is lacking, probably because of the high temperature of the body. In artificial cultures a much better growth is observed at 22° C. than at 37° C.

The differentiation of pathogenic sporotricha into distinct species by means of fermentative reactions is stated by Meyer to be impossible. This investigator believes that the American strains of pathogenic sporotricha are best classified as one species, Sporothrix schenckii-beurmanni.[1]

Cases of human sporotrichosis are reported in this country and in Europe in increasing numbers, but it is not certain whether the infection is becoming more common or whether it is more frequently recognized.

Sporotrichosis in domesticated animals, particularly horses, is often noted in certain parts of the United States; but transference of the disease from animals to man is observed only in rare instances.[2]

[1] K. F. Meyer: Jour. Infect. Dis., 1915, 16, p. 399.
[2] K. F. Meyer: Jour. Amer. Med. Assoc., 1915, 65, p. 579.

CHAPTER XXXI

THE PARASITIC PROTOZOA

Introductory.—The Protozoa, the lowest group of the animal kingdom, are especially distinguished from the higher animals, or Metazoa, by the fact that, as a rule, each organism consists of but a single cell. They range in size from organisms scarcely larger than bacteria to organisms several centimeters in length. It is hardly possible at present to define accurately the class of Protozoa. The group is very extensive and heterogeneous, and includes some organisms of great simplicity and some of extraordinary elaboration of structure. The life-cycle of many protozoa is complex and involves the alternation of sexual and asexual phases. Study of the life-cycle is rapidly changing our conception of relations within the group and of the group itself.

At the present time most protozoölogists recognize about twenty-four well-defined species of protozoa parasitic in man; some of these organisms produce fatal or very serious diseases. The interest in parasitic protozoa is shown by the appearance in the last few years of three general texts,[1] and a large number of monographs dealing with single groups.

Many species are parasitic upon various animals and plants. Several widespread and serious plagues of domestic animals are due to invasion of the body by various protozoan parasites, and the part played by protozoa in causing diseases of mankind, especially in the tropics, is far more important than was at one time suspected. The following table, taken largely from Wenyon,[2] gives a classification of the protozoa with examples selected from among the parasites of man:

[1] Hegner and Taliaferro: "Human Protozoölogy," New York, 1924, pp. 597; Craig: "A Manual of the Parasitic Protozoa of Man," Phila., 1926, pp. 569; Wenyon: "Protozoölogy," London, 1926, pp. 1563.

[2] Wenyon: "Protozoölogy" p. 159.

Sub-phylum Plasmodroma. Movement by changeable protoplasmic processes called pseudopodia or by permanent whip-like organs, flagella.

Class Rhizopoda. Predominating phase is ameboid, locomotion by pseudopodia. Ex: *Endameba.*

Class Mastigophora. Predominating phase is flagellated. Locomotion by flagella. Ex: *Trypanosoma.*

Class Cnidosporidia. Exclusively parasitic forms which are frequently ameboid. Dissemination by characteristic spores provided with "polar capsules" from which long filaments can be extruded. Many fatal epidemic diseases of cold-blooded vertebrates and insects are caused by members of this group. No parasites of man are known to belong to it, but *Sarcocystis*, whose affinities are very little understood, is sometimes placed in it.

Class Sporozoa. Exclusively parasitic forms which typically reproduce by schizogony and after syngamy has occurred produce sporozoites enclosed in resistant oöcytes. Ex: *Plasmodium.*

Sub-Phylum Ciliophora. Movement by means of cilia. Typically with nuclear dimorphism—macronucleus and micronucleus. Ex: *Balantidium.*

Mouth Endamebæ.—The discovery of an ameba from the tartar of the teeth by Gros[1] in 1849 probably is the first description of any parasitic ameba. Recently much interest has been aroused by the finding of the same ameba in the disease of the gums known as pyorrhea alveolaris, and several investigators have considered that the endamebæ were the cause of this affection. The ameba of the gums (*Endameba gingivalis*) resembles *E. histolytica* (Fig. 138). It is probably transferred from mouth to mouth in the ameboid form as no cysts are known. It is extremely common in the mouth and most authors believe it to be a harmless commensal. Its abundance in diseased conditions such as pyorrhea is probably due simply to the abundance of food at such times.

Intestinal Amebæ

The Ameba of Dysentery—Endameba Histolytica.—Ameboid organisms have been found abundantly in the intestinal discharges of persons suffering from a peculiar form of chronic dysentery especially common in the tropics, but by no means rare in temperate countries. In the majority of autopsies on these cases the ulcerations of the intestinal wall contain amebæ in large numbers, and the histologic appearances at these points indicate an active invasion of the tissues (Fig. 136). Amebæ are also found in the internal organs, notably in the liver, where large abscesses highly characteristic of this form of dysentery occur in from 20 to 25 per cent. of all cases. These abscesses harbor amebæ in great abundance,

[1] Gros: Bull. Soc. Imp. Nat., Moscow, 1849, 22 (1 part), p. 549.

and no other micro-organism has been constantly demonstrated in these situations culturally or microscopically.[1] The serum of patients suffering from this form of dysentery does not agglutinate the bacillus of epidemic dysentery (p. 348). The peculiarity of the symptoms and lesions, the localization of the amebæ in the intestinal ulcers and in the characteristic liver abscesses, and the absence of specific bacteria and other parasitic organisms have caused this form of dysentery to be generally ascribed to the pathogenic activity of the ameba. The presence of amebæ in the stools

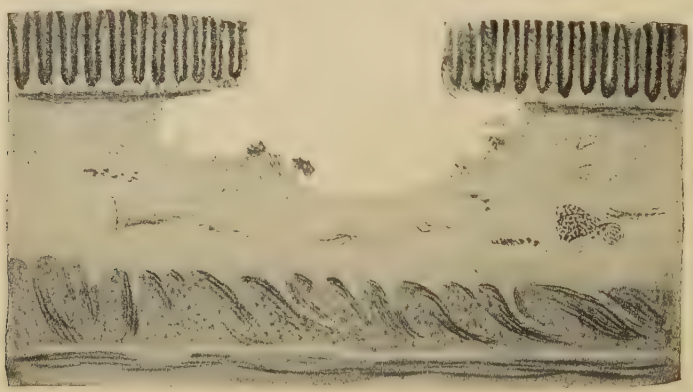


Fig. 136.—Section of an intestinal ulcer (Kartulis, in Kolle and Wassermann).

seems to have been first noted in 1870 by Lewis,[2] whose description was so inadequate that the species cannot be identified from his paper. His co-worker Cunningham,[3] in 1871, gave a much better description, from which the forms can be identified as being chiefly *E. coli*. The first observations on *E. histolytica* were probably made by Lösch in 1875.[4] The animal experiments of Lösch, the discovery of amebæ in sections of the intestine by Koch,[5] the extensive investigation of dysentery in Egypt by Kartulis,[6] and the comprehensive study of the clinical and pathological characteristics of the disease

[1] The abscesses are not, however, so commonly sterile bacterially as was at one time asserted. Staphylococcus aureus, B. coli, and some other bacteria are found accompanying the amebæ with considerable frequency.

[2] Lewis: Appendix to Ann. Rept. of Sanitary Commissioner with Govt. of India. Calcutta, 1870.

[3] Cunningham: Ann. Rept. of Sanitary Commissioner with Govt. of India. Calcutta, 1871, pp. 141–243.

[4] Lösch: Arch. path. Anat., 1875, 65, p. 196.

[5] Koch and Gaffky: Arb. a. d. kaiserl. Gesundh., 1887, 3, p. 1.

[6] Kartulis: Virchow's Archiv. f. path. Anat., 1886, 105, p. 521.

by Councilman and Lafleur[1] went far to establish the independent nature of the malady and its connection with the presence of amebæ.

Characteristics and Life-cycle.—The large ameboid and tissue-invading form of *E. histolytica* usually measures 20–30 μ in diameter and moves by the extrusion of clear hyaline pseudopodia (Figs. 137 and 138). The nucleus is characteristic of the genus and exhibits a thin peripheral layer of chromatin granules and a small central mass of chromatin known as the karyosome. The active forms reproduce by binary fission. So far as known they obtain their food partly by absorption and partly by the ingestion of red blood cells and tissue elements. No proof has been given that they ever

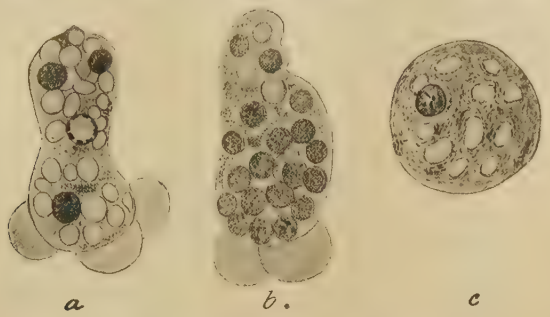


Fig. 137.—*Endameba histolytica* (Kruse and Pasquale): *a* and *b*, Amebæ as seen in the fresh stools, showing blunt ameboid processes of ectoplasm. The endoplasm of *a* shows a nucleus, three red corpuscles, and numerous vacuoles; that of *b*, numerous red corpuscles and a few vacuoles; *c*, an ameba as seen in a fixed film preparation, showing a small rounded nucleus; × 600.

eat bacteria in the body, although they do feed on them in culture. The primary site of the infection is the large intestine where the ameba produces typical undermined ulcers extending into the mucosa, submucosa or even muscular layers of the intestinal wall. These ulcers always open into the lumen of the intestine and are constantly discharging amebæ. In symptomless carriers of the parasite the amebæ so discharged become rounded and sluggish, and stop ingesting food. Such amebæ are much smaller than the tissue-invading forms and, as they are preparing for encystment, are known as precystic forms. Eventually as the precystic forms move down the intestine they secrete a clear thin cyst wall about themselves and are known as uninucleate cysts. These cysts are spherical and usually measure from 5 to 20 μ in diameter. They do not change in size, but as they pass down the intestine the nucleus

[1] Councilman and Lafleur: Johns Hopkins Hospital Rept., 1891, 2, p. 395.

divides into 2 and then 4 nuclei. The quadrinucleate cyst is the mature form and passes out of the body with the feces. The younger

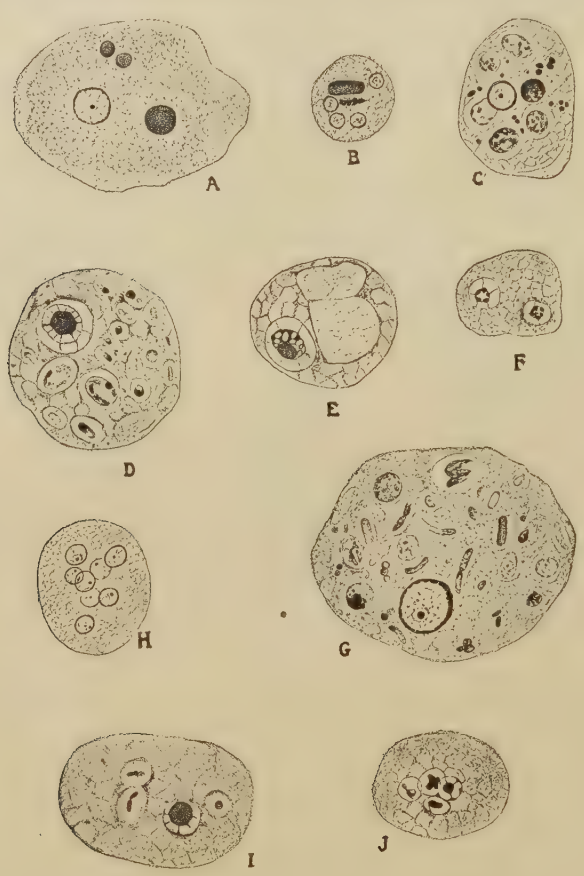


Fig. 138.—The amebæ living in man. A, large tissue-invading form of *Endameba histolytica* containing three red blood cells. B, mature quadrinucleate cyst of the same containing two chromatoids. C, active ameboid form of *Endameba gingivalis*. D, active ameboid form of *Iodameba williamsi* containing many intestinal bacteria. E, cyst of same showing large double vacuole which in life was filled with glycogen. F, ameboid form of *Dientameba fragilis* containing two nuclei. G, large active ameboid form of *Endameba coli* containing intestinal bacteria and debris. H, mature eight-nucleate cyst of same. I, ameboid form of *Endolimax nana*. J, mature quadrinucleate cyst of same. A, B, C, G, and H × about 1300 (after Dobell). D, E, F, and J × 3000 (after Taliaferro and Becker). I × 3000 (after Taliaferro in Hegner and Taliaferro's "Human Protozoölogy," courtesy of The Macmillan Company).

stages of the cysts usually contain masses of glycogen and often also blunt rounded masses of chromatic material known as chromatoids.

Both of these are gradually reabsorbed, but mature quadrinucleated cysts may retain one or more chromatoids.

The mature quadrinucleate cyst is the only infective form and when swallowed probably hatches in the small intestine. Eventually the progeny set up ulceration in the large intestine.

The outline just given portrays the probable course of events in the symptomless carrier. If the intestinal ulcers are very extensive the patient suffers from acute dysentery. In this condition the tissue-invading forms are flushed out of the intestine before they have time to encyst. The carrier who is passing cysts is therefore the only person who can disseminate the infection. Every gradation is found between symptomless carriers and patients suffering from acute dysentery. All the evidence indicates, however, that by far the majority of infected persons are carriers that never suffer from symptoms. This explains the fact that, while in the United States approximately 10 per cent. of the population is infected, there are comparatively few cases of dysentery. In the tropics the proportion of infected persons exhibiting symptoms is probably greater than in the United States.

Although the primary site of the infection is probably always the large intestine, the amebæ sometimes set up secondary sites of infection. The commonest of these is the liver, as has been noted. In addition a number of cases have been described in the spleen and even in the brain. Apparently authentic instances of the occurrence of amebæ in the urine have been noted, and one case in which *E. histolytica* was found in the vas deferens and tubules of the epididymis. Kofoid and Swezy[1] and Kofoid, Boyers, and Swezy[2] believe that they have demonstrated *E. histolytica* in the bone lesions of Ely's non-bacterial or second type of arthritis and in the lymphatic glands of Hodgkin's disease respectively. In all of these secondary sites the amebæ live essentially as they do in the deeper portions of the intestinal ulcers. Since the amebæ in such sites cannot complete their life-cycle, such invasions probably represent *cul de sacs* from which they cannot escape. Both carriers and patients with symptoms are liable to such secondary invasions.

The life-history of the dysentery amebæ outside the human body is essentially unknown, but there is the best evidence, epidemiolog-

[1] Kofoid and Swezy: Jour. Amer. Med. Assoc., 1922, 78, p. 1602.

[2] Kofoid, Boyers and Swezy: Jour. Amer. Med. Assoc., 1922, 78, p. 1604.

ically, that the disease is spread chiefly by raw foods and water. The contamination of food may occur either indirectly by means of sewage or polluted water or directly by a carrier or convalescent. Flies may conceivably be agents in the transmission of infection; amebic cysts have been found in the feces of these insects. A very thorough description and full bibliography of the parasitic amebæ of man is given by Dobell.[1]

Non-pathogenic Amebæ of the Intestine.—Besides *E. histolytica* there are at least four other species of ameba living in the human intestine: *Endameba coli, Endolimax nana, Iodameba williamsi* and *Dientameba fragilis.* These forms have much the same life-cycle as *E. histolytica,* except that they are restricted to the lumen of the alimentary canal, never invade the tissues, and, as far as is known, do not give rise to any untoward symptoms. Whereas *E. histolytica* feeds on red blood cells and tissues, these forms never eat tissue elements, but ingest bacteria, yeasts and intestinal debris. *Dientameba fragilis* is a rare species. The other three are differentiated from *E. histolytica* by many features, chief of which are the nature of the food inclusions in the ameboid stages, the structure of the nucleus, and the size, shape and nuclear number of their cysts. The various details are shown in Fig. 138.

Recently Boeck[2] has studied the incidence of the different intestinal amebæ in 8029 inhabitants of the United States with the following results:

	Number Infected	Percentage Infected	Estimated Percentage Infected
E. histolytica	333	4.1	8–10
E. coli	1596	19.6	36–46
E. nana	1060	13.2	25–31
I. williamsi	404	5.0	10–15

The figure given under "estimated percentage infected" is probably the true index of infection as it is the figure arrived at after making allowance for the number of infections probably missed by his routine methods. Similar conclusions have been obtained by Dobell[3] after compiling the data for the British Isles.

Cultivation of Intestinal Amebæ.—The cultures obtained by the earlier investigators who attempted to cultivate the parasitic amebæ were all probably free-living species which represented the

[1] Dobell: "The Amoebae Living in Man," London, 1919, pp. 155.

[2] See Boeck and Stiles: Bull. 133, Hyg. Lab., Washington, 1923.

[3] Dobell: Med. Res. Council, Special Report Series, No. 59, London, 1921.

progeny of cysts of those species that had passed unchanged through the intestinal canal or were air contaminations. Cutler[1] (1918) was undoubtedly dealing with *E. histolytica,* but most investigators feel that his work is open to a different interpretation from the one he placed on it. The first undoubted cultivation of a parasitic species was that of Barret and Smith[2] (1923, 1924) who grew *E. barreti* from the turtle in a 1:10 dilution of inactivated human serum in 0.5 per cent. saline. This work was fully confirmed by Taliaferro and Holmes in 1924.[3] The first undoubted cultivation of *E. histolytica* is the work of Boeck and Drbohlav, 1925.[4] These investigators used solid egg and blood agar slants covered with Locke's solution containing serum or egg albumin. Cultures were kept at 30°–37° C. and subcultures were made every two or three days. There are many modifications of the original Boeck-Drbohlav medium for which the student is referred to such books as Wenyon's[5] which describe in detail standard methods of cultivation for the protozoa.

At the present time it seems that Barret and Smith's method is suitable for many amebæ of cold-blooded vertebrates and that Boeck and Drbohlav's technic can be applied to most amebæ of warm-blooded animals. All of the amebæ of man except the rare *Dientameba fragilis* have been cultivated on the original Boeck and Drbohlav's medium or modifications of it. Recently Craig[6] has succeeded in growing *E. histolytica* in a 1:8 dilution of inactivated human serum in .85 per cent. saline, which is manifestly similar to Barret and Smith's method. All cultures of parasitic amebæ contain numerous bacteria upon which the protozoa feed.

Intestinal Flagellates of Man.—The finer structure of the intestinal flagellates is so difficult to study that there is still considerable disagreement among various authorities in regard to the exact number of species, their classification and the structure and arrangement of certain organelles. There are probably at least five species —*Giardia lamblia, Chilomastix mesnili, Trichomonas hominis,*

[1] Cutler: Jour. Path. and Bact., 1918, 22, p. 22.

[2] Barret and Smith: Amer. Jour. Hyg., 1923, 3, p. 205; 1924, 4, p. 155.

[3] Taliaferro and Holmes: Amer. Jour. Hyg., 1924, 4, p. 160.

[4] Boeck and Drbohlav: Proc. Nat. Acad. Sci., 1925, 2, p. 235; Amer. Jour. Hyg., 1925, 5, p. 371.

[5] Wenyon: "Protozoölogy," p. 1296.

[6] Craig: Amer. Jour. Trop. Med., 1926, 6, p. 461.

Embadomonas intestinalis and *Tricercomonas intestinalis*, of which the first three are the best known and are undoubtedly world-wide in distribution.

Giardia (Fig. 139), which is a peculiar bilaterally symmetrical flagellate, possesses two nuclei. The active forms live in the upper

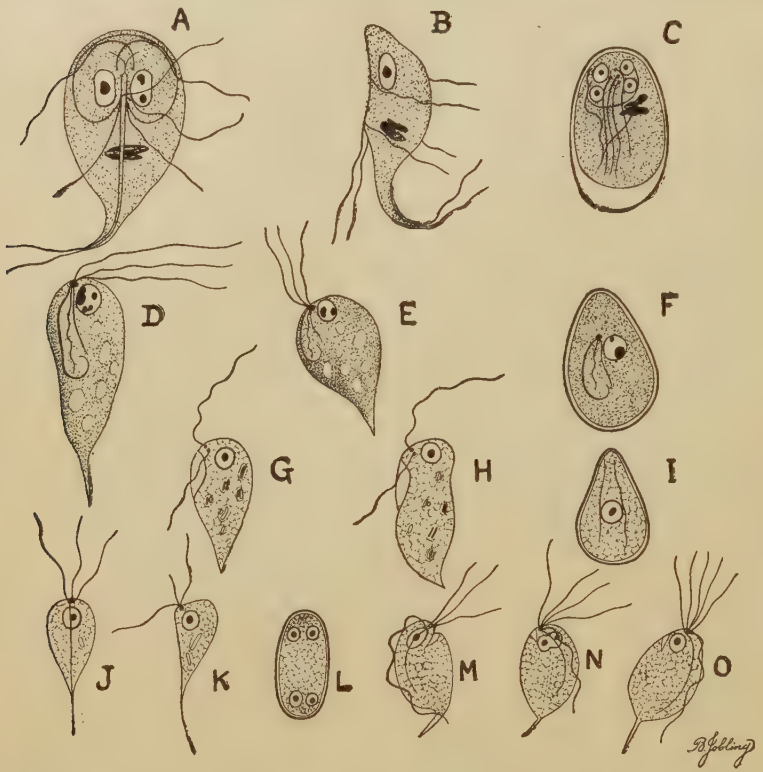


Fig. 139.—The flagellates of the human intestine (× 2000). A–C, *Giardia lamblia*, free and encysted forms. D–F, *Chilomastix mesnili*, free and encysted forms. G–I, *Embadomonas intestinalis*, free and encysted forms. J–L, *Tricercomonas intestinalis*, free and encysted forms. M–O, *Trichomonas hominis*, forms with three, four, and five flagella (from Wenyon, Proceedings of Royal Institution of Great Britain, March 3, 1922).

portion of the small intestine, whereas the other species all live in the large intestine. With the exception of *Trichomonas* these flagellates all form cysts, which are the infective forms. The cysts are the forms ordinarily encountered in the stools, as the free flagellates are not washed out except during diarrheic conditions. Cysts of *Trichomonas* have never been described, and it seems probable from

the work of Hegner[1] on this form, that unlike what is known of all other intestinal protozoa, infection can result from the ingestion of the active free forms.

The question of the pathogenicity of the intestinal flagellates of man is a matter of considerable controversy. Most suspicion is directed toward *Giardia* and *Trichomonas*. Wenyon[2] found *Trichomonas* not only in the lumen of the glands but breaking through the glandular cells and distributed in the interglandular tissue in sections from an autopsy. He feels that it is impossible to decide whether this invasion is an indication of pathogenicity. There is even the possibility of postmortem invasion.

The Trypanosomes

The Trypanosomes (Gr. τρυπᾶν, to bore) are a group of free-swimming protozoa occurring in the blood-plasma and other body-fluids of mammals, birds, reptiles and other animals. Although trypanosomes were observed in the blood of salmon as long ago as 1841, it was not until the investigations by Bruce in 1894 upon the dreaded tsetse-fly disease of Zululand that the pathogenic qualities of these organisms came to light. At the present time at least ten well-defined diseases of domestic animals and two diseases of man are known to be due to various species of trypanosomes. In addition, other trypanosomes have been found in the blood of a very large number of different animal species, in which they seem to occasion little or no harm to the host organism. The trypanosomes of fish, reptiles, and amphibia seem to be transferred from one animal to another by the bite of the leech, in which they are known to be present in the proboscis-sheath. Mammalian trypanosomes are transmitted commonly by fleas and various species of biting flies. An enormous number of trypanosomes have been described. Laveran and Mesnil[3] have given a classical description of the group.

The trypanosome found in the common rat is an example of the non-pathogenic group. It may also serve as a morphologic type:

Trypanosoma lewisi.—This parasite infests the blood of wild rats, and in many localities is found in from 25 per cent. to 100 per cent. of all rats captured. In examining a fresh blood-film the rapidly

[1] Hegner: Amer. Jour. Hyg., 1926, 6, p. 593.

[2] Wenyon: Jour. Trop. Med. and Hyg., 1920, 23, p. 125.

[3] Laveran and Mesnil: "Trypanosomes and Trypanosomiases," 1912, Paris, pp. 1000.

moving trypanosome readily attracts attention by the disturbance of the blood-corpuscles in its immediate neighborhood. At one end is a single, long, free whip or flagellum. The trypanosome usually moves with the flagellum foremost, and in consequence the flagellate end is designated as the anterior end. The posterior extremity of Tr. lewisi is rather sharply pointed, this being one of the characteristics that distinguishes it from other trypanosomes. The body of the trypanosome itself is spindle-shaped, and a fin-like structure extends from a point near the posterior end of the body to the base of the whip. This fin is the so-called undulating membrane; it plays, perhaps, a more important part in locomotion than the flagellum itself.

When the trypanosome is stained by the Romanowsky method, a small roundish body is seen near the sharp posterior end. This is the so-called parabasal body. Associated with it is the blepharoplast, which is rarely seen in Romanowsky preparations and which is connected with the base of the flagellum. This parabasal body and the blepharoplast together are often termed the kinetoplast. The flagellum is connected with the blepharoplast and extends along one side of the protozoön to the anterior end, and it is supposed that the blepharoplast functions as a motor center. In the rat trypanosome the nucleus is situated near the center of the body. The length of Tr. lewisi is about 30 μ or approximately three and one-half times the diameter of a red corpuscle.

When the cell is about to divide, it lengthens, becomes somewhat stouter, and at the same time the nucleus draws near to the blepharoplast. Usually the blepharoplast divides first, then the parabasal body and still later the nucleus. The body of the cell then undergoes fission and gives birth to a young trypanosome, which may at once become detached from the mother cell. In all cases division is longitudinal. Transverse division never occurs.

In some cases division takes place after a fashion analogous to the segmentation of the malarial parasite, resulting in the formation of a rosette, composed of from four to eight small trypanosomes with their flagella pointing outward (Fig. 140). Such rosette formation is apparently due to a retardation of the division of the protoplasm, the nuclei and the blepharoplasts dividing first without corresponding separation of new individuals. Reproduction by cell division is limited to the first ten days of the infection, after

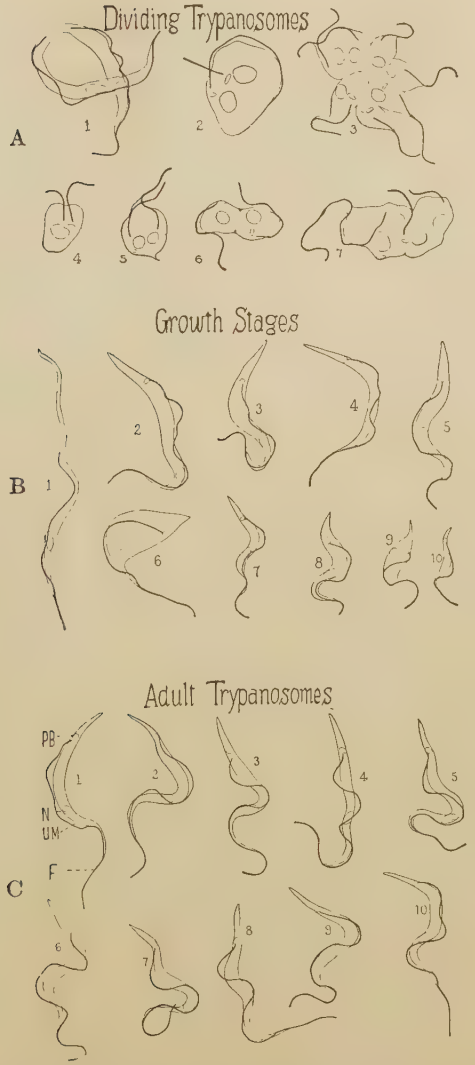


Fig. 140.—Trypanosoma lewisi. A. Dividing trypanosomes and "rosettes" such as are found at the beginning of an infection during the height of reproductive activity. B. Growth stages taken from the fourth day of an infection before reproduction and growth has been inhibited. C. "Adult" forms such as are found after the tenth day when all reproduction and growth is inhibited. F, flagellum, N, nucleus, P. B., parabasal body, U. M., undulating membrane. × 1000. (A, after Coventry; B and C after Taliaferro from Hegner and Taliaferro's "Human Protozoölogy," courtesy of The Macmillan Company.)

which the trypanosomes live in the blood as non-reproducing "adults." Taliaferro[1] has shown that this cessation of reproduction is due to the formation of a reaction-product in the rat which inhibits the cell division of the parasites, but which does not kill them. The typical pathogenic trypanosomes do not develop rosettes in the blood, but reproduce by binary fission throughout the course of the infection. No evidence of true conjugation in trypanosomes has yet been discovered.

Injection of a small quantity of rat blood containing trypanosomes into a healthy rat produces an infection in about three to four days. As a rule, the trypanosomes disappear from the blood of inoculated animals within two or three months after infection, but in exceptional cases they may persist much longer. Unlike the pathogenic trypanosomes, Tr. lewisi is peculiar to one kind of animal—the rat—and cannot be transferred to any other species. White rats, as well as the common wild rats, are susceptible. It has never been definitely shown that Tr. lewisi is ever fatal to rats, but such infections have been described.

When a rat has once become free from the parasite, it is found to have acquired an active immunity and cannot be reinfected. The blood of rats that have received a number of injections of trypanosoma possesses protective properties, and the injection of immune serum from such animals will prevent infection in normal rats. Transmission of the rat trypanosome from infected animals to healthy ones can take place through the agency of insects, such as rat fleas, lice, etc. In the flea the trypanosomes multiply chiefly in the lower portions of the alimentary tract. Infection of the rat through the agency of the flea does not occur by means of the proboscis, and the trypanosomes are absent from the flea's salivary glands. The infective dejecta of the flea may be swallowed by the rat in the act of licking the fur, or the puncture made by the insect may be contaminated with flea dejecta.

In 1903 Novy and MacNeal[2] succeeded in cultivating the rat trypanosome in pure culture outside of the body. The culture-medium employed is a blood-agar composed of equal parts of defibrinated rabbit blood and nutrient agar. The agar is melted and

[1] Taliaferro: Jour. Exper. Med., 1924, 39, p. 171.

[2] Novy and MacNeal: "Contributions to Medical Research," dedicated to V. C. Vaughan, Ann Arbor, 1903, p. 549.

cooled to about 50° C., after which the rabbit blood is added and thoroughly mixed.

"The tubes thus prepared are allowed to set in an inclined position, after which they are at once inoculated. It is essential that the surface of the medium be moist and soft, and if this is not the case, the tubes should be placed in an upright position until some water of condensation accumulates at the bottom. The initial culture usually requires a week or more, although not infrequently fairly rich growths may be obtained in three or four days" (Novy).

By the use of this medium Novy and his associates have kept trypanosomes under cultivation for several years, carrying them in this time through nearly one hundred generations. The trypanosome of nagana (see below, Tr. brucei) has also been cultivated artificially in a similar manner.[1] Trypanosomes found in birds can be grown with especial ease by this method.

The more definitely pathogenic species of trypanosomes may now be briefly considered.

Trypanosoma evansi.—A disease of horses and camels characterized by remitting fever, anemia, and edema is common in India, where it is known as *surra*. This affection has been shown to be due to the presence of a species of trypanosome. The same or a very similar form of trypanosomiasis also occurs in other parts of Asia and in Africa. In the Philippines it has caused much loss among horses and cattle. Experiments have shown conclusively that flies (*Stomoxys*, *Tabanus*) are able to transmit the disease, provided they bite within a few hours after feeding on the infected host. These insects are probably mere mechanical carriers.

Trypanosoma brucei.—The early explorers of the continent of Africa found their movements greatly interfered with by a disease that affected their beasts of burden, and that destroyed horses, mules, and oxen in large numbers; dogs also are affected. This disease was attributed by the natives to the bite of an insect called the tsetse-fly, and European observations served to confirm the existence of such a connection. The tsetse-fly disease is commonly called by the native name nagana. It has been observed especially in Zululand, but occurs likewise in other parts of Africa. Nearly all of the large mammals seem susceptible to natural or experimental infection.

[1] Novy and MacNeal: Jour. Infect. Dis., 1904, 1, p. 1.

In the blood of animals suffering from nagana Bruce[1] discovered the trypanosome which is called by his name. This trypanosome has been obtained in cultures by Novy and MacNeal,[2] and extended experiments have been carried out with the isolated organisms.

Certain tsetse-flies, namely, *Glossina morsitans*, and others of this genus, seem to be the only insects whose bite is able to convey the nagana infection, since ordinary biting insects that have fed on infected animals are not able to communicate the disease to healthy subjects. It is possible that the infection is sometimes transferred mechanically by the biting tsetse-fly, but there is also evidence that a cyclical development of the parasite occurs in the insect's body. Except for the first few hours after biting, when mechanical transference is possible, the fly is not infective until about the eighteenth day. It may remain infective for at least twelve weeks and probably much longer. In animal experiments about one wild fly out of 500 is able to produce infection when caught. There is reason to believe that the parasite exists in the blood of big game in parts of Africa, and that the fly becomes infected from these animals and transmits the disease to horses and cattle. As Bruce expresses it, the reservoir of the disease is found in the wild animals. It is said that the extermination of the larger wild herbivora in parts of southern Africa has rendered the tsetse-fly disease relatively uncommon.

A form of "sleeping sickness" in man, formerly supposed to be due to a specific trypanosome different from *Tr. gambiense* (p. 569) and for a time designated as *Tr. rhodesiense*, is now believed by some investigators to be due to *Tr. brucei*. Like the nagana, the human infection is transmitted by the bite of *Glossina morsitans*. This disease is especially prevalent in Nyasaland and Rhodesia. It is more rapidly fatal than the sleeping sickness of the Congo, but does not occur in epidemics.

Trypanosoma equinum.—A disease known as mal de Caderas, which is prevalent in parts of South America and attacks especially horses, is due to a trypanosome which resembles in general size and form the other pathogenic trypanosomes, but is easily differentiated by the apparent absence of a parabasal body: as a matter of fact, this structure is present, but is very inconspicuous. Mal de Caderas

[1] Bruce: Preliminary Report on the Tsetse-fly Disease or Nagana in Zululand, Durban, 1896.

[2] Novy and MacNeal: Jour. Infect. Dis., 1904, 1, p. 1.

is characterized especially by paralysis of the hindquarters and by the almost complete absence of the edemas which are usually present in nagana and surra.

Trypanosoma theileri.—This trypanosome of cattle is unusually large (30 μ to 65 μ in length), about the size of the bird trypanosomes. Trypanosoma theileri, like Tr. lewisi, is both non-pathogenic and seems to be limited to one host, since inoculations of animals other than cattle have failed. It is apparently transmitted by hippoboscid and tabanid flies.

Trypanosoma equiperdum.—A disease of horses met with in parts of Europe, and also reported from the western parts of the United States and Canada, is caused by a trypanosome very similar to the other pathogenic forms. This disease has long been known by the name of dourine, or mal du coit; it is usually of a chronic character, the animal becoming gradually paralyzed and dying, as a rule, within from two to ten months. A noteworthy feature of the disease is that it is spread, so far as known, exclusively by sexual congress, and not by biting insects. Experimentally the disease can be readily communicated to the horse, ass, dog and rabbit.

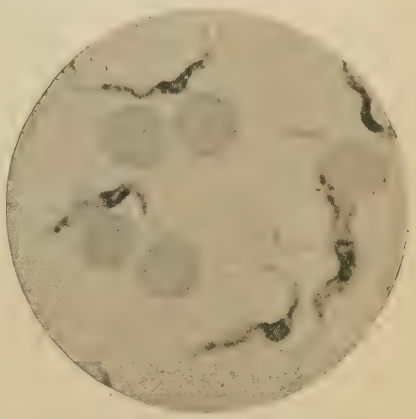


Fig. 141.—*Trypanosoma gambiense.* The parasite of sleeping sickness; × about 1400 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

Trypanosoma gambiense.—In addition to these definite infections of the lower animals, a form of human trypanosomiasis is known which prevails extensively among the natives of tropical Africa and also affects Europeans. This is the terrible disease known as sleeping sickness. It has been estimated that between 1896 and 1906 from 400,000 to 500,000 natives in the Congo region perished from this pestilence.

The parasite, *Trypanosoma gambiense*, resembles very closely the trypanosomes of surra and nagana. The disease is conveyed by the bite of a fly, *Glossina palpalis* (Fig. 142), belonging to the

same genus as the tsetse-fly that carries the nagana of Zululand. The early experiments seemed to indicate that transmission was merely mechanical and was due to the direct injection of contaminated blood by a fly that had recently (within three days) fed on an infected animal. The experiments of Kleine and others, however, show that after a period of about twenty-five days the fly again becomes capable of communicating the infection, and is hence probably a true host for the trypanosome. The salivary gland emulsions are infective.

Fig. 142.—Tsetse-fly, *Glossina palpalis* ($\times 3\frac{1}{4}$), the carrier of the trypanosome of sleeping sickness (Adami).

Sleeping sickness is characterized by two stages, in the first of which the trypanosomes are found in the blood, although always in small numbers; the pulse and respiration are accelerated, but in general the symptoms are mild. Glandular enlargements are an early and constant feature, and the trypanosomes are practically always found in the enlarged glands. After a variable length of time the second stage of the disease is entered upon; this is characterized by the symptoms which have given rise to the name of sleeping sickness. The patient becomes dull and apathetic, great weakness of the limbs develops, and emaciation is usually extreme. Finally, a condition of coma ensues and is followed by death.

Some kinds of monkeys (macaques) may be successfully inoculated, and develop symptoms very similar to those seen in man.

The larger domestic animals are rather refractory to inoculation, but dogs and cats are quite susceptible. White rats are also readily infected. Monkeys and dogs have been found infected under natural conditions. Certain wild animals (*e. g.*, antelopes) appear to serve as reservoirs whence the parasites may be derived in the absence of man. The destruction of game animals has, consequently, been advocated as a measure for preventing the spread of the disease. A relatively small number of *Glossinæ* captured in a wild state are infective.

In parts of Africa the disease is terribly prevalent. Dutton and Todd[1] found that in some villages from 30 to 50 per cent. of the population were infected. So far as known, untreated sleeping sickness is invariably fatal. The treatment of the human disease is fairly successful if begun in the early stages. Among the most used drugs is the organic arsenical, atoxyl, which was introduced by Thomas in 1905. This has been supplemented to a great extent with tartar emetic (sodium or potassium antimonyl tartrate) and antimony trioxid. Therapy of the disease bids fair to be revolutionized by the introduction of tryparsamide (the sodium salt of *N*-phenylglycineamide-*p*-arsonic acid), which was first synthesized and studied at the Rockefeller Institute for Medical Research,[2] and Bayer 205, which was first introduced in Germany by Haendel and Joetten[3] and Mayer and Zeiss.[4] Tryparsamide is even effective in some of the advanced stages.[5] None of these drugs is effective in Rhodesian sleeping sickness or in Chagas' disease.

Various attempts have been made to immunize animals artificially against trypanosomiasis. Success so far has been obtained almost solely with killed trypanosomes, and the results have been so variable that no attempt has been made to apply the method to human beings.

Trypanosoma cruzi.—Another disease of man attributable to trypanosomes is "Chagas' disease," a Brazilian form of trypano-

[1] Dutton and Todd: "First Report of the Expedition to Senegambia," 1902. "Trypanosomiasis," Liverpool School of Tropical Medicine, Memoir II, Liverpool, 1903.

[2] See series of papers by Jacobs and Heidelberger and Brown and Pearce: Jour. Exper. Med., 1919, 30, pp. 411, 417, 437, 455, 483. Also Pearce: Ibid. 1921, 34, Suppl. No. 1.

[3] Haendel and Joetten: Berlin klin. Woch., 1920, 57, p. 821.

[4] Mayer and Zeiss: Arch. Schiffs. u. Trop.-Hyg., 1920, 24, p. 257

[5] Abbatucci: Ann. Méd. et Pharm. Colon., 1926, 24, p. 83.

somiasis, transmitted by a bug belonging to the family of Reduviidæ (*Triatoma megista*). The saliva and the excreta of this bug are infective. The incidence is almost entirely among children. The thyroid gland is hypertrophied, and the liver always enlarged. One interesting feature of this parasite is that much of its life-cycle in man is in the form of an intracellular leishmania-like stage (Fig. 143). In this stage the trypanosome is morphologically indistinguishable from a true Leishmania.

Leishmania.—A disease known as kala-azar, dum-dum fever, or tropical splenomegaly, occurring in parts of India as a house-

Fig. 143.—Leishmania-form of *Trypanosoma cruzi* in human heart muscle. × 3700 (Taliaferro in Hegner and Taliaferro's "Human Protozoölogy," courtesy of The Macmillan Company.)

hold or family malady, and at one time regarded as a malarial cachexia, has been ascribed by several observers to a trypanosome-like parasite.[1] This disease is of very long duration and is highly fatal. In 1903 Leishman[2] described certain peculiar bodies found in the spleen of a patient suffering from this form of protracted fever, and a similar observation was made independently by Donovan[3] shortly after. Subsequent investigations by Rogers[4] and others have shown that the "Leishman-Donovan bodies" pass through certain developmental phases in infected blood drawn from the body and mixed with sodium citrate. These cultural forms (Fig. 144) are very similar in structure to the flagellates of the genus *Herpetomonas*, which occur in the gut of various insects, and immediately betray their flagellate affinities. The name *Leishmania*

[1] See Rogers, Milroy Lectures, Brit. Med. Jour., 1907, 1, pp. 427, 490, 557.
[2] Leishman: Brit. Med. Jour., 1903, 1, p. 1252.
[3] Donovan: Brit. Med. Jour., 1903, 2, p. 79.
[4] Rogers: Lancet, 1905, 1, p. 1484.

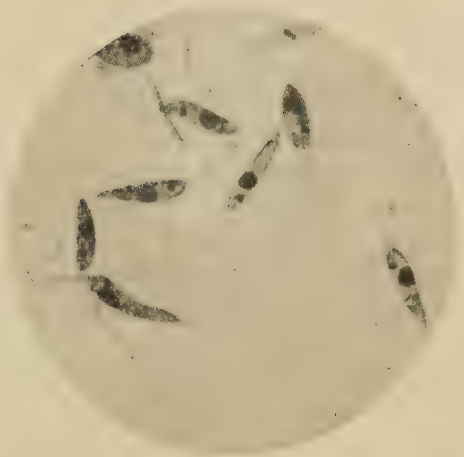
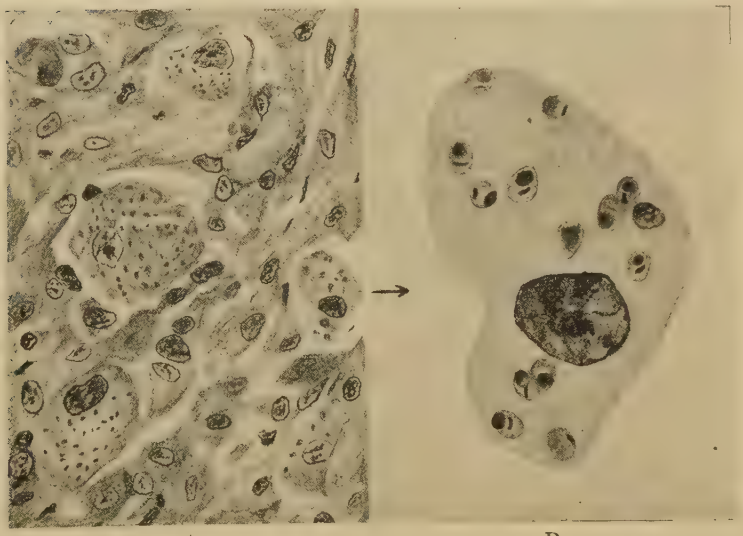


Fig. 144.—*Leishmania donovani.* Flagellated forms, from a culture. Wright's stain. × 1800 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).



A B

Fig. 145.—*Leishmania donovani* in the human spleen. A, section of spleen showing five endothelial cells infected with the parasites. B, one cell enlarged to show structure of the parasites. A × about 550; B × 2500 (from Taliaferro in Hegner and Taliaferro's "Human Protozoölogy," courtesy of The Macmillan Company).

donovani has been given to this microörganism (Fig. 145). An excellent description and bibliography of the leishmanioses of man may be found in the monograph of Laveran.[1]

Various investigators have attempted to incriminate different biting insects as the vector of the disease. At the present time a large mass of evidence has accumulated, indicating that the sand fly *Phlebotomus* is the transmitter of both *Leishmania donovani* and *Leishmania tropica.*

By the use of appropriate technic, positive cultures may be obtained from the peripheral blood in at least as many cases as from spleen punctures.[2]

The infection is connected with particular locations—house or soil—and can be successfully combated by destruction of infected dwellings, removal from contaminated soil, and segregation. In certain tea-gardens in India the application of these measures has caused the disease practically to disappear.

The intravenous injection of sodium antimonyl tartrate is considered a specific curative treatment (Rogers) and is used in this and other leishmanioses.

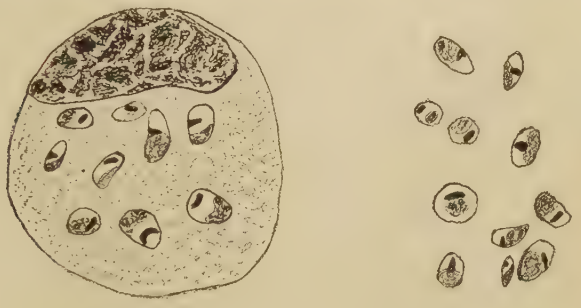
It was formerly supposed that in the Mediterranean area the disease attacked only young children and was due to a specific parasite (*L. infantum*). It is generally conceded now that the Indian and Mediterranean diseases are identical and attack both adults and children.

Delhi Boil.—Micro-organisms very similar to those found in kala-azar have been found in the affection known as oriental sore, Delhi boil, or tropical ulcer. Although apparently seen by Cunningham in 1885, they were first accurately described and pictured by J. H. Wright.[3] The name *Leishmania tropica* (Fig. 146) is commonly used for these organisms. The disease is inoculable from one individual to another. The sand fly *Phlebotomus papatasii* is the probable transmitter of the disease. Although there is a close morphologic resemblance between the Wright bodies and Leishman bodies, the very different nature of the pathologic conditions with which they are associated would seem to indicate that the two organisms are not identical. A few cases of Oriental sore and of

[1] Laveran: "Leishmanioses." Paris, 1917.
[2] Young, C. W. and Van Sant, Helen: Jour. Exper. Med., 1923, 38, p. 233.
[3] Wright, J. H.: Jour. Med. Res., 1903, 10, p. 472.

kala-azar (contracted in the old world) have been observed in the United States.[1]

A similar disease occurs in South and Central America and is known by various names in different localities. The parasite *L. braziliensis* is probably distinct from *L. tropica* as shown by the recent serological studies of Noguchi[2] and by its tendency to involve the mucosa. The flagellate stage of the organism has been observed, and animals have been successfully inoculated from human cases.



A B

Fig. 146.—*Leishmania tropica* from a case of oriental sore. A, macrophage containing nine parasites. B, a clump of eleven extracellular parasites taken from the same smear. Note the presence of a nucleus and parabasal body in each parasite. × 2000 (from Taliaferro in Hegner and Taliaferro's "Human Protozoölogy," courtesy of The Macmillan Company).

The Malarial Parasites

Introductory.—The writings of antiquity contain frequent mention of the clinical manifestations of malaria, but although the disease was known for centuries, surprisingly little real information was acquired concerning its significant etiologic features until the last quarter of the nineteenth century. Perhaps the most important of the early observations upon the natural history of malaria consisted in noticing that the disease prevailed especially in some localities and not in others. In fact, the geographic and topographic distribution of the malarial fevers is so peculiar that it excited the languid curiosity of many ancient peoples and caused them to set afloat various surmises to account for such distribution, which, however, were in the main unprofitable and fruitless.

[1] Faber, H. K., and Schussler, J.: Jour. Amer. Med. Assoc., 1923, 80, p. 93.

[2] Noguchi: International Conference on Health Problems in Tropical America. United Fruit Co., 1924, p. 455.

The advent of bacteriology caused the study of malaria to be taken up from the new point of view, and at first several enthusiastic workers reported the discovery of certain bacteria which they deemed to bear a causal relation to the disease. These reputed findings failed to be confirmed. It was not until November 6, 1880, that the true malarial parasite, a protozoön of the class Sporozoa, was discovered by Laveran,[1] a French military surgeon stationed in Algiers. In 1885 Golgi[2] described in detail the life-history of the parasite of quartan fever, and later brought forward strong evidence that the parasites of tertian and estivo-autumnal fever could be morphologically differentiated from the quartan form. Golgi also showed that the malarial chill or paroxysm always coincides with the sporulation in the blood of a brood of parasites.

Following this work a number of investigators suggested the transfer of the disease by mosquitoes. Among these Manson,[3] because of his previous studies on *Filaria* in the mosquito, suggested the experimental problem of testing the mosquito theory of malaria to Ross. Ross first demonstrated the mosquito cycles in bird malaria, but it remained for Grassi[4] and his co-workers to give the first clear-cut proof of the cycle in the human disease.

The Asexual Development of the Malarial Parasites.—In the human body the malarial micro-organism passes through certain regular phases of development within the red blood-corpuscles. The parasite in its youngest recognizable stage appears to be attached to the corpuscle as a small, glassy, oval, rounded or ring-like body with ameboid movements, which proceeds to burrow slowly into the substance of the corpuscle, increasing in size at the latter's expense. After attaining its maximum development the mature and full-grown parasite, which varies in size according to the species, undergoes a segmentation of its cell-substance, giving rise first to the appearance of a rosette or mulberry-like body within the corpuscle, and leading eventually to the formation of small rounded bodies known as merozoites. The young merozoites are liberated by the ultimate disintegration of the corpuscle, and on

[1] Laveran: Bull. Acad. de méd., 1880, ser. 2, 9, p. 1346.

[2] Golgi: Arch. per le Sci. med., 1886, 10, p. 109; Ztschr. f. Hyg., 1891, 10, p. 136.

[3] Manson: Brit. Med. Jour., 1898, 2, p. 849.

[4] Grassi, Bignami and Bastianelli: Atti. R. Accad. Lincei, Rendie, 1898, 5 ser. 8, 21.

being set free fasten themselves to new red corpuscles and begin once more their cycle of development. The setting free of the merozoites is practically coincident with the appearance of the chill or malarial paroxysm, and the remarkable periodicity of the malarial attack is thus conditioned by the time necessary for the development of the protozoön. Only the asexual mode of development here outlined has been observed to occur in the human body.

Bass and Johns[1] have succeeded in cultivating the malarial parasite (tertian and estivo-autumnal types) outside of the body. The asexual cycle so cultivated *in vitro* does not differ from the same

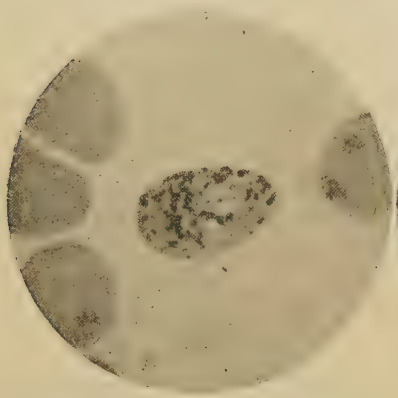


Fig. 147.—*Plasmodium vivax.* Three-quarters-grown parasite. × 1800 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

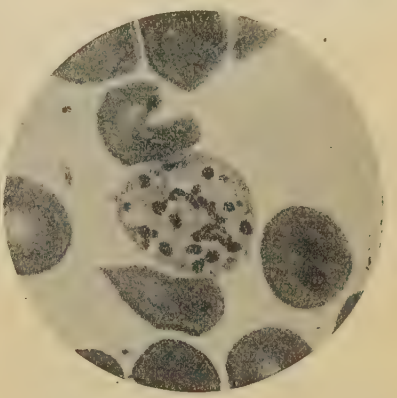


Fig. 148.—*Plasmodium vivax.* Sporulating parasite. × 1800 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

cycle growing *in vivo*. The most rapid growth is obtained at a temperature of 40° to 41° C. By centrifugalizing the blood sufficiently to remove the leukocytes the parasites may be transferred from tube to tube of blood-cells and carried through several generations. Unless the leukocytes are thus removed, phagocytosis of the parasites will occur when the latter escape from the red blood-cells at the time of segmentation. Human red blood-cells are necessary and there is no evidence that the parasites can be grown outside these cells.

Morphology of the Different Varieties of the Malarial Parasite.—Three varieties of the malarial parasite can be distinguished, and most observers are agreed that the three forms are biologically distinct. Each is found associated with a type of fever possessed

[1] Bass and Johns: Jour. Exper. Med., 1912, 16, p. 567.

of a clinical individuality: (1) tertian malarial fever, (2) quartan malarial fever, (3) estivo-autumnal fever. The tertian fever is the more common malarial fever of temperate countries, is rarely fatal, and is readily amenable to quinin. The estivo-autumnal

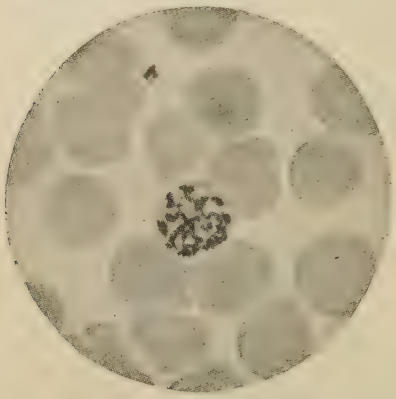


Fig. 149.—*Plasmodium malariæ.* Three-quarters-grown parasite. × 1500 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

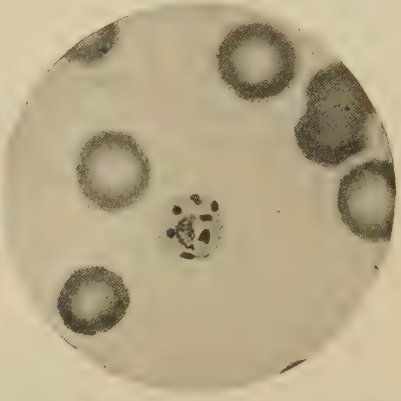


Fig. 150.—*Plasmodium malariæ.* Sporulating parasite. × 1200 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

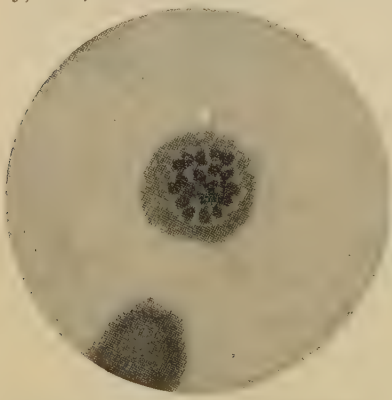


Fig. 151.—*Plasmodium falciparum.* Sporulating parasite. × 1800 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

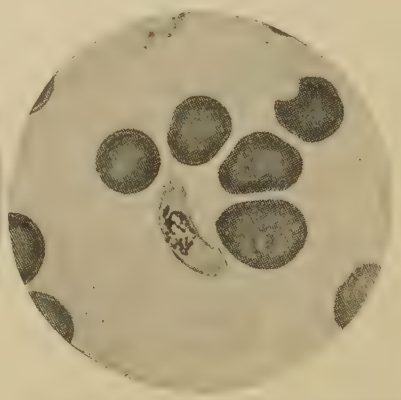


Fig. 152.—*Plasmodium falciparum.* A macrogametocyte. The female crescent form or gamete. × 1200 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

fever is more prevalent in the tropics, is more deadly, more irregular in course, and relatively refractory to treatment. Quartan is comparatively rare in most localities.

1. *The Tertian Parasite* (*Plasmodium vivax*).—The time required for the asexual development of the tertian parasite is forty-eight

hours. The paroxysms accordingly appear on alternate days, or, according to the Roman method of reckoning time, the first attack is followed by the recurrent attack on the third day, whence the name of tertian fever. The full-grown tertian parasite is quite large, and may even reach a diameter nearly double that of the corpuscle. The young parasites show active ameboid movement. Twelve to twenty-four merozoites—about sixteen on an average—are produced.

2. *The Quartan Parasite* (*Plasmodium malariæ*).—The quartan parasite completes its asexual development in seventy-two hours, the malarial attacks taking place therefore at intervals of three days. The young parasite manifests a less active ameboid movement than the tertian form, and the pigment is of a more coarsely granular character (Fig. 149). The size of the adult quartan parasite does not exceed that of the red corpuscle. The number of the merozoites produced is usually eight, but may range between six and fourteen (Fig. 150).

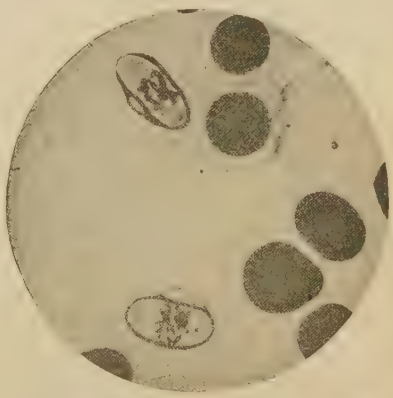


Fig. 153.—*Plasmodium falciparum.* A microgametocyte. The male gamete or crescent form. × 1200 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

3. *The Estivo-autumnal Parasite* (*Plasmodium falciparum*).—Most students of malaria believe that there is a single type of estivo-autumnal parasite, but some, notably Craig, believe that two varieties exist, one the quotidian form and the other the tertian form. Undoubtedly the fever, which occurs at the time of schizogony and which is determined by the length of the asexual cycle, is variable and may occur every 48 hours or every 24 hours. Some of this however, may be due to double infections, the schizogony of which takes place on alternate days.[1] *P. falciparum* differs from the other

[1] Some cases of malarial fever in which the attacks recur at twenty-four-hour intervals may be due not to a specifically distinct parasite, but to infection with more than one brood of parasites. Thus a double tertian infection in which brood A matures on the first and third day, brood B on the second and fourth day, and so on, or a triple quartan infection, or a double quartan together with a single tertian, may give rise to the quotidian type of fever (Golgi).

two species in that besides the small ring stages the asexual development takes place in the vessels of the deeper organs and not in the peripheral blood. These asexual forms are irregularly distributed over the body and tend to select definite organs. Thus with a cerebral localization there are predominantly cerebral symptoms, etc. The schizont gives rise to from 8 to 24 merozoites (Fig. 152). The gametocytes occur in the peripheral blood and are differentiated from the other species by their crescent shape (Figs. 153 and 154).

Fig. 154.—Diagrammatic representation of the differences between the three species of malarial parasites of man. Row A, *Plasmodium vivax* of tertian fever. Row B, *P. malariæ* of quartan fever. Row C, *P. falciparum* of estivo-autumnal fever. No. 1 in each row represents a red cell containing trophozoites in the ring stage; No. 2, schizonts; No. 3, merozoite formation; and No. 4, macrogametocytes. (From Hegner in Hegner, Cort and Root's, "Outlines of Medical Zoölogy," courtesy of The Macmillan Company.)

The great majority of the "pernicious" and fatal cases of malaria are due to the estivo-autumnal parasite. Tertian infections are said to be much more common than estivo-autumnal. The estivo-autumnal fevers are more irregular in character than the ordinary tertian or quartan types.

The table on page 581 shows the main differences between the three forms of parasite.

The Sexual Phase of the Parasite.—The malarial parasite, as has been stated, passes only its asexual phase of development within the human body. A complicated sexual phase is consummated within the body of the mosquito. After the sexual develop-

ment is completed, the parasite may again enter the body of its mammalian host, borne along with the fluid injected by the mosquito in the act of biting, and forthwith embark upon a new asexual cycle. Zoölogically considered, man is the intermediate host of the malarial parasite, and the mosquito (Anopheles) its true host. The elucida-

DIFFERENCES BETWEEN THE THREE FORMS OF MALARIAL PARASITES

Parasites of:	Asexual cycle completed in:	Effect on red blood-corpuscle	Size and shape of mature asexual parasite or schizont. Number of merozoites formed	Sexual form or gametocyte
Tertian fever....	Forty-eight hours	Corpuscle swollen, nearly normal in color, and after Romanowsky's stain granules in infected cells (Schüffner's dots) outside of parasite.	One and one-half times the diameter of the corpuscle, mulberry-shaped. Twelve to twenty-four young parasites formed by segmentation.	Both gametocytes spherical or ovoid in shape. Male gametocyte (spheres) about two-thirds as large as female gametocyte, which is about one and one-half times as large as a normal red corpuscle. Male with a single centrally placed nucleus and female with a single excentrically placed nucleus.
Quartan fever..	Seventy-two hours	Corpuscles normal in size; darker in color than normal; no pigment in cell outside of parasite.	Approximately the size of the corpuscle, daisy-shaped. Six to fourteen merozoites formed.	Both gametocytes spherical or ovoid in shape. Female gametocyte somewhat smaller than normal red cell and male about three-quarters size of red cell. Positions of nuclei the same as in tertian.
Æstivo-autumnal fever.	Twenty-four hours to forty-eight hours	Corpuscles greenish, about normal in size; partially decolorized; after stain granules of pigment (Maurer's dots) in cell outside of parasite.	Always much smaller than the corpuscle, usually about one-fourth the diameter. Six to twenty-four merozoites.	Gametocytes crescent-shaped, with their longest diameter greater than diameter of normal red cell. The male gametocyte broader and shorter than female. The nucleus of both centrally placed, but that of the male more dispersed.

tion of the remarkable relations subsisting between man, the mosquito, and the malarial parasite is due to Ronald Ross,[1] an English army officer, stationed in India at the time of his investigations.

The steps that led up to this discovery are of peculiar interest. The occasional occurrence in the blood taken from malarial patients of crescent-shaped bodies, "flagellated forms," and other deviations from the strict asexual type received the easy interpretation by some writers of a mere degeneration phenomenon. The observation, however, that the flagellated bodies were not seen in freshly drawn blood, but only appeared after the blood had been exposed for a short time to air, caused some investigators (Manson[2]) to adopt the hypothesis that the advent of these bodies marked an abortive attempt on the part of the parasite to enter upon the sexual stage. As a corollary to this hypothesis, the assumption appeared warranted that the sexual phase of the parasite was passed in the body of some suctorial insect, and suspicion eventually fastened on the mosquito.[3] As a result of this reasoning, Ross undertook his researches in India. Other investigations were pointed in the same direction. In 1897 MacCallum[4] found that the so-called flagella sometimes seen attached to the parasites of bird malaria were in reality male sex cells, which entered the larger spherical female cells of the avian malarial parasite, a process presumably one of fertilization.

Ross, who also studied bird malaria, obtained convincing evidence in the first place that the malarial parasite of birds underwent an intricate sexual development within the body of a certain kind of mosquito (Culex), and found that the infection could be communicated to normal birds by means of the bite of infected mosquitoes. He further observed that the human malarial parasite could not develop in the body of Culex, but did develop in the stomach of another variety of mosquito (Anopheles). Finally (August, 1897) he recognized that certain pigmented cells found in the stomach of mosquitoes (Anopheles) that had been fed with the

[1] Ronald Ross: Indian Med. Gaz., 1898, 33, pp. 14, 133, 401, 448.

[2] Manson: Brit. Med. Jour., 1894, 2 pp. 1252, 1306; "Tropical Diseases," London, 1900.

[3] King had previously advanced arguments in support of the mosquito-malaria hypothesis in a paper that seems at the time to have attracted little attention (Popular Science Monthly, Sept., 1883).

[4] MacCallum: Lancet, 1897, 2, p. 1240.

blood of human malarial patients were developmental phases of the parasite.[1]

Conclusive demonstration that malaria could be transmitted by the bite of infected mosquitoes was afforded by Bignami[2] at Rome, and also in an especially striking manner by Manson in London. The latter investigator, who had never previously suffered from malaria and who lived in England, a country free from indigenous malaria, allowed himself to be bitten by some forty mosquitoes that had been shipped from Rome after sucking blood from a case of tertian malaria. An attack of the disease followed, and typical tertian parasites were found in the blood.

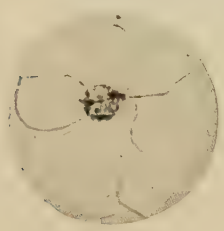


Fig. 155.—Microgametocyte, showing "flagella" which are the microgametes (Koch).

Fig. 156.—Fertilization, tertian parasite: 1, Extrusion of nuclear substance by macrogamete; 2, macrogamete with divided nucleus; 3, entrance of microgamete into the macrogamete; 4, zygote (Schaudinn).

The sexual development of the parasite within the body of the mosquito may be briefly outlined. In the body of man some of the merozoites, instead of developing into schizonts, become sexual parasites or gametocytes. The precise period in the asexual cycle at which the gametocytes first appear in the blood of an infected person is not known. After being taken into the stomach of the mosquito the nuclei of the gametocytes undergo a reduction division and give rise to gametes. The male cell (microgametocyte) develops four to eight delicate, hyaline filaments (microgametes) (Fig. 155); one of these enters one of the large spherical and granular macrogametes (female cell), and effects a true fertilization (Fig. 156). The copula (zygote, oökinete) resulting from their union penetrates the stomach wall of the mosquito, where it becomes encysted (oöcyst), and increases greatly in size, so that the stomach wall of

[1] Ross: Brit. Med. Jour., 1898, 1, pp. 550, 1575, 1607.
[2] Bignami: Lancet, 1898, 2, pp. 1461, 1541.

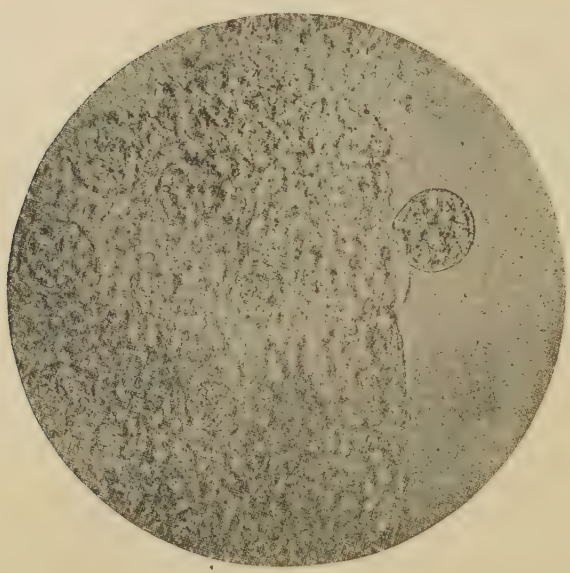


Fig. 157.—Malarial zygotes in stomach wall of the mosquito. High power (Manson).

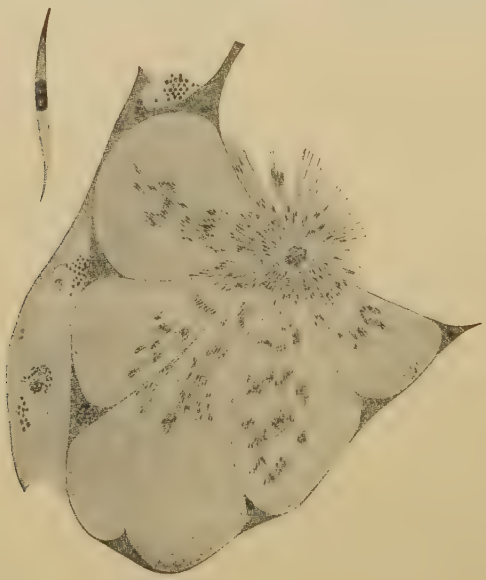


Fig. 158.—Section of salivary gland of a mosquito, Anopheles. Sporozoites (Grassi).

an infected mosquito becomes studded with numerous[1] wart-like protuberances (Fig. 157). Small spherical bodies (sporoblasts) are formed in the interior of the oöcyst. Finally these sporoblasts by internal divisions give rise to myriads of delicate filamentous bodies (sporozoites) which eventually are liberated by the rupture of the oöcyst and are carried by the lymph to all parts of the body of the mosquito, with the possible exception of the ovary.

As has so often happened in the course of parasitic evolution, the malarial parasite is restricted, so far as known, to certain definite hosts. There is no evidence that the parasites of human malaria can invade the red corpuscles of any other mammalian species, with the possible exception of some of the higher apes. It is also true that the genus of mosquito known as Anopheles is the only kind of mosquito that has been shown capable of harboring this specific protozoön.[2]

The main differences between the genus Anopheles[3] and the closely related and very common genus Culex are shown in the accompanying figures (Fig. 159). The chief generic distinction is based upon the length of the palpi, which in the female Anopheles are as long as the proboscis and in the female Culex are always much shorter. Many species of Anopheles are easily distinguished from Culex by the possession of spots on the wings,[4] but this is not

[1] Grassi estimates that five hundred may develop in the stomach walls of a single insect.

[2] Not all species of Anopheles furnish an equally good soil for the propagation of the malarial parasite. This is notably the case with one species common in India and the Philippines (*A. rossi*), which, although experimentally capable of conveying infection, is very rarely, if at all, infected under natural conditions. James found that, among 736 individuals of *A. rossi* caught in native huts, not one was infected, although about one-half of the native children in the neighborhood harbored the malarial parasite, and another less common species of Anopheles (*A. culicifacies*) was infected in proportions varying from 4.6 to 8.7 per cent. (Ztschr. f. Hyg., 1903, 43, p. 218). *A. febrifer* is the chief carrier of malaria in the Philippines.

The three common American anophelines are all known to be susceptible to infection with malarial parasites: *A. punctipennis* and *A. crucians* with *Plasmodium vivax* and *Plasmodium falciparum; A. quadrimaculatus* with these and also with *Plasmodium malariæ*. The last named mosquito is probably the most important factor in malarial dissemination in this country.

[3] Some authorities have split up the original genus of Anopheles mosquitoes into ten or more new genera, but general agreement concerning the classification of these organisms has not yet been reached.

[4] This is true of all but one of the seven species reported from the United States, and this exception, *A. barberi*, is a very rare species.

Fig. 159.—Comparison of Culex and Anopheles. Eggs, larvæ (note position), position of insects at rest, wings, heads showing antennæ and palpi (Kolle and Hetsch).

a universal distinction. A further difference between the two genera consists in the position assumed while at rest, the body of Culex, as a rule, being parallel to the surface on which the mosquito is resting, while that of Anopheles forms a more or less acute angle with the surface. In Anopheles, furthermore, the head, thorax, and abdomen form one straight line, whereas in the resting Culex the thorax and abdomen form an angle with the head and proboscis (Fig. 159). Some Anopheles assume the resting position of Culex. Thus one of the worst malaria carriers of India rests in the Culex attitude. The eggs and the larvæ of the two genera can be readily distinguished.

The habits and distribution of Anopheles explain many of the most characteristic features in the epidemiology of malaria. Many species of Anopheles are almost wholly nocturnal in their habits, rarely biting by day, hence the greater liability of contracting malaria during the night hours. The great abundance of Anopheles in certain localities and at certain seasons accounts for certain long-observed peculiarities in the geographic and seasonal distribution of the disease. The connection of malarial fevers with marshy localities, the prevalence of the disease in country districts rather than in cities, the often striking exemption of persons on board vessels lying off a malarious coast, the frequent breaking out of the disease in consequence of extensive soil excavations and disturbance of natural water-courses—such as occurs, for example, in railroad construction—all these idiosyncrasies of malarial fever can be explained through the creation or maintenance of breeding-grounds for Anopheles, or through the opportunities afforded Anopheles for access to malarial patients and subsequently to uninfected persons. Many other factors influence the causation of malaria because they affect the insect host of the malarial parasite rather than the parasite itself. Summing up the matter of malarial infection, it can be said that the proximity of a malarial patient is never a source of danger unless Anopheles mosquitoes occur in the immediate environment, and, conversely, the Anopheles mosquito is not to be feared unless there are present in the neighborhood persons bearing the malarial parasites in their blood.

Prophylaxis.—Accepting the theory that malaria is conveyed only through the bite of an infected mosquito, there are at least three ways in which the spread of the disease may be combated:

1. The parasite when within the human body may be injured or destroyed by means of the systematic and continued administration of quinin, thus preventing the infection of mosquitoes and breaking this link in the chain of causation. This is the method advocated especially by Koch,[1] and in certain regions, as in the case of several small islands near the coast of New Guinea, it has been employed with great success in stamping out the disease. The free distribution of quinin to the poor in Italy has been fol-

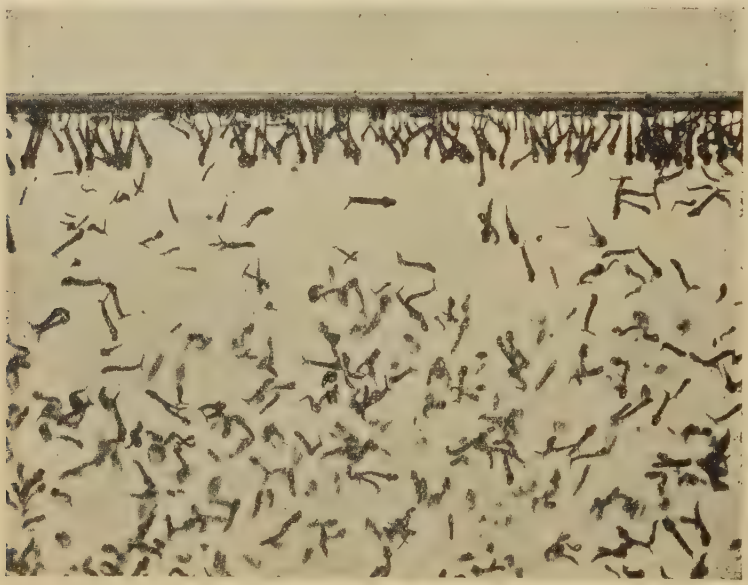


Fig. 160.—Mosquito "wigglers"—larvæ and pupæ—in the water. Life size (Underwood).

lowed by a marked reduction in the mortality from malaria (from 15,865 in 1900 to 3619 in 1910). It is clear that when the available supply of malaria parasites is cut off, the bite of Anopheles can no longer convey infection, since there is absolutely no evidence that Anopheles can become infected with the malarial protozoön in any other way than by sucking blood from an infected human being. It is the opinion of Koch and others, who have devoted much study to the conditions in tropical countries, that from the point of view of community-infection, malaria can be effectively dealt with only in this way. The cost of the quinin necessary for such an undertaking is of course great, and this has been urged as an objection

[1] Koch: Deut. med. Wchnschr., 1899, 25, p. 69.

against the method, but in a few instances the total outlay for quinin has been actually diminished as compared with the previous consumption of this drug, owing to the systematic mode of procedure that has been introduced. Koch has pointed out that in tropical countries it is almost exclusively the native children that harbor the malarial parasite; the adult natives, owing apparently to the severe infections to which they have been subjected in childhood, are nearly all immune. Quinin acts most energetically upon the young parasite, and in accordance with this fact has been found to be most efficacious in the tertian and quartan fevers when administered in a single large dose during the decline of temperature, that is to say, shortly after the young parasites have been set free in the blood. In the estivo-autumnal fever, where less regularity exists, and different broods of parasites come to maturity at different times, smaller doses at frequent intervals have been found more useful.

The Subcommittee on Medical Research of the National Malaria Committee recommends the following method of treatment not only for enabling the patient to avoid a relapse, but for preventing the transmission of the infection to others:

For an acute attack, 10 grains quinin sulfate, administered through the mouth, three times a day for a period of at least three or four days. This dosage should be followed by a treatment consisting of 10 grains taken every night before retiring, for a period of eight weeks. For infected persons who have not shown acute symptoms only the eight weeks' treatment, without the preliminary dosage, is necessary.

For children the doses vary according to the age of the patient, as follows:

Age of Child	Dose of Quinin, Grains
Less than 1 year	0.5
1 year	1
2 years	2
3 and 4 years	3
5, 6, and 7 years	4
8, 9, and 10 years	6
11, 12, 13, and 14 years	8
15 years or over	10

One of the most interesting recent developments in the therapy of malaria is the introduction of Plasmochin by the German workers.

Plasmochin is a synthetic quinolin derivative[1] and although present data are not sufficient to draw definite conclusions, the preliminary work on the treatment of bird malaria,[2] induced malaria in general paresis[3] and natural cases[4] indicates that it will be a very valuable adjunct in the treatment of the disease. It is supplied as Plasmochin tablets containing 0.02 gm. and as Plasmochin compound tablets containing 0.01 gm. Plasmochin and 0.125 gm. quinin sulphate. The preliminary experience of the United Fruit Company indicates that, of the two, Plasmochin compound gives fewer toxic symptoms and is the more effective therapeutically. In this form they consider it "an exceedingly valuable therapeutic agent."[5]

2. A second method that has been ardently advocated as suitable for the extirpation of malaria consists in directly attacking the mosquito host. This mode of conducting the campaign against malaria has been especially urged by Ross and other English authorities. This can best be done by attacking the larval stages in the breeding pools. Where the cost is not prohibitive the best method, because of its permanency, is to eliminate the breeding site. This is an engineering problem and consists of draining, filling and altering streams so that they become unsuitable as breeding places. Where these permanent measures are too expensive or impractical, the larvæ may be killed by poisons. The older poisons are the so-called "contact" variety and consist of oils or various prepared larvacides. Recently much attention has been given to "stomach" poisons. These are substances which will float on the surface film and be eaten by the larvæ. They are efficient only for anopheline mosquitoes which are surface feeders and are not effective for Culex which feeds below the surface. One of the first poisons to be used was paraform. In the United States the use of Paris green has been particularly developed. This substance is mixed with road dust and strewn over the body of water to be treated in concentrations so low that it does not make the water unsuitable for the consumption of people and large animals. This method promises to be the cheapest and most satisfactory for general malaria control. In special localities use can sometimes be made of the natural

[1] Horlein: Beihefte, Arch. f. Schiffs-Trop.-Hyg., 1926, 30, p. 305.
[2] Roehl: *Ibid.*, 1926, 30, p. 311.
[3] Sioli: *Ibid.*, 1926, 30, p. 319.
[4] Mühlens: *Ibid.*, 1926, 30, p. 325.
[5] XV Ann. Rept. Med. Dept. United Fruit Co., Boston, 1926, p. 66.

enemies of mosquito larvæ such as "top minnows." The latter method is peculiarly adapted to the control of the mosquito carrying yellow fever.

At first the control of malaria by mosquito reduction was efficient in the temperate regions of the earth, but it was generally believed that in the tropics, with the great profusion of species, the problem was absolutely hopeless. Recent investigations have shown, however, that in any given locality there are only a few—often only one—species of Anopheles actually carrying malaria to any great extent. By ascertaining which species are the dangerous carriers and finding out their peculiar habits it has been found possible to concentrate control measures on these forms. By these methods malaria in the tropics is being effectively controlled.

3. The spread of the disease can be checked in some measure by the consistent use of mosquito netting, mosquito-proof houses, and other mechanical devices for shielding malaria patients against the bite of mosquitoes, thereby preventing the infection of Anopheles. In the same way healthy individuals may be safeguarded from the bite of infected mosquitoes. That a high degree of protection can be afforded by suitable precautions against mosquito attack is proved by the experience of Sambon and Low, who spent several months during the malarial season in a carefully constructed hut in the Roman Campagna. These investigators breathed the same air and drank the same water as the other inhabitants of this malaria-stricken region, but, owing to the precautions taken in the matter of retreating at nightfall to their mosquito-proof sleeping apartments, they remained entirely exempt from the disease. This form of mechanical prophylaxis has been applied on a large scale in Italy to the homes of railway employees, customs officials, and others compelled by their vocation to dwell in malarial regions, and is said to have given "résultats vraiment magnifiques" (Celli). In Formosa not a single case of malaria developed among 115 soldiers in screened barracks (September to December), while during the same period 251 malarial infections occurred among 717 soldiers not so protected. It is not, however, always possible for tropical residents so to order their lives as to protect themselves constantly from the bites of mosquitoes, and although the liability to malarial infection may be somewhat lessened by the conscientious employment of mosquito netting and other protective devices, the danger

cannot be altogether avoided. In regions where malaria is not very common, and particularly in temperate climates, much can doubtless be done to prevent the extension of the disease by educating the community to the desirability of thoroughly screening malaria patients and shielding them so far as possible against mosquito bites.

The inference is clearly justified that no one of the three methods advocated for prophylactic purposes can be reasonably neglected. Protection against mosquito bites, abatement of the number of mosquitoes in a given locality, and diminution in the number of persons harboring the malarial parasite will all surely lead to a reduction in the prevalence of malaria, and any improvement will be cumulative.

Other Hemosporidia.—Various malarial parasites infecting birds of several genera have been described and are quite common and of cosmopolitan distribution. The life-history of the Plasmodium of birds has been worked out with some degree of completeness and is very similar to that of the human form. The sexual phase of the avian plasmodium is accomplished in the body of the mosquito, but in a different genus (Culex, not Anopheles). Canaries frequently succumb to artificial infection with the avian plasmodium, while sparrows, which are often found infected under natural conditions, die but rarely.

Various protozoa, parasitic within blood-corpuscles, are also found in frogs, turtles and other cold-blooded animals. Lankesterella, a very common form occurring in the frog, is perhaps the best known of these. The sporozoites of this form occur in the blood of the frog and are probably transferred without further development to other frogs by a leech.

The Piroplasmas

The parasitic protozoa now commonly known under the generic name of *Piroplasma* (Lat., *pirus*, a pear) or *Babesia* were first discovered in 1889 by Theobald Smith[1] in the blood of cattle suffering from a disease known as Texas fever, tick fever, or bovine malaria. The disease known in South America as *La Tristeza* is identical with this affection. Other piroplasma-like organisms have since been found in the blood of various animal species. Most

[1] Smith, Theobald: Bull. 1, Bureau of Animal Industry, Washington, D. C., 1893.

investigators are agreed that definite generic differences exist, and accordingly have established new genera, such as *Theileria* (the parasite of African East Coast fever), *Nuttallia* (a widely distributed parasite causing a disease of horses), and *Nicollia* (in the gondi, a North African rodent). The relationship of many parasites provisionally placed in this group is entirely uncertain.

The prevention of piroplasma infection has been successfully accomplished by freeing the animals from ticks by dips containing arsenic, accompanying this treatment by removal from tick-infested pastures. Preventive measures based on the elimination of the tick in this way have been very successful in combating Texas fever. Intravenous infection of trypan-blue has a curative effect upon certain piroplasma infections in cattle and dogs, but not upon others, for instance, the parasite of East Coast fever in cattle.

The parasites have not been cultivated and neither monkeys nor rabbits have been infected by inoculation with human blood containing the parasite.

Fig. 161.—*Babesia bigemina* in blood from kidney (Smith).

Babesia bigemina.—Texas fever is characterized especially by destruction of the red blood-corpuscles, accompanied by hemoglobinuria; the spleen is greatly enlarged and the liver extensively affected. The disease, which is peculiar to cattle, is common in the southern United States, and occurs also in South America, parts of Europe and Africa. In addition, a similar parasite, *B. bovis*, occurs in cattle in all parts of Europe and *B. mutans* occurs in Europe, Asia, Africa and Australia, but not in America. A remarkable feature in the natural history of Texas fever is that cattle raised in a disease-ridden district may to all appearances be entirely healthy, and yet when imported into uninfected territory, transmit the disease to susceptible animals.

The piroplasma is found in the blood of infected animals, where it occurs within the red corpuscles. In the acute form of the disease the parasites appear as pear-shaped bodies, usually two in number in each corpuscle, with their pointed ends in juxtaposition (Fig. 161). Ameboid movements are observed in certain stages. The parasites are seen in fresh preparations with difficulty, but they stain distinctly with alkaline methylene-blue and other dyes.

That some connection existed between the cattle tick (*Boöphilus annulatus*) and Texas fever was long suspected by practical stockmen, but such a connection was first demonstrated by the work of Smith and Kilborne. So far as known, infected ticks are the only means by which the disease is spread. Ticks that mature upon the bodies of animals containing piroplasmas in their blood *never attack another animal,* but drop to the ground after the usual fashion of

Fig. 162.—Cattle tick, *Boöphilus annulatus.* Sexually mature female after last moult (Smith).

these insects, and lay their eggs. The eggs hatch in about three weeks, and the young ticks in the larval and nymphal stages crawl upon the bodies of cattle that may be grazing in the tick-infected pasture (Figs. 162 and 163). It has been experimentally proved that the bite of these young ticks, descended from piroplasma-infected mothers, is able to communicate the infection. The adult

Fig. 163.—*Boöphilus annulatus;* eggs and young tick, just hatched (Smith).

insects themselves do not pass from infected cattle to healthy ones, but transmit the disease only indirectly by way of their progeny. Smith and Kilborne have shown that cattle from the permanently infected territory, though otherwise healthy, carry the piroplasmas of Texas fever in their blood. These facts explain many singular features in the epidemiology of the disease, such as the breaking out

of Texas fever in the northern herds upon the introduction of apparently healthy cattle from the South; the sickening of northern cattle imported into the South and not brought into contact with southern cattle, but pastured in tick-infested fields; and the inability of cattle sick with Texas fever to communicate the disease unless they are at the same time infested with ticks.

Theileria parva.—A disease of cattle in Africa which is in some respects similar to Texas fever has been attributed by Koch and others to a parasite of the piroplasma group. The organism in question is smaller than *Babesia bigemina* and presents other distinct points of difference, so that it is placed in a separate genus. The sickness, known as Rhodesia fever, or East Coast fever, is spread by tick bite and resembles Texas fever in many respects. Mixed infections with the East Coast fever and Texas fever parasites seem to be not uncommon and have caused much confusion. Hemoglobinuria is absent in East Coast fever, and the number of red corpuscles in the peripheral circulation is not appreciably diminished. The mortality is high (80 to 90 per cent.). Unlike Texas fever, East Coast fever appears to leave animals recovering from it incapable of infecting ticks. At least five species of the tick *Rhipicephalus* are capable of transmitting the infection. The parasite is not passed on from the parent tick to the egg, but may be transmitted from one developmental stage to another, from larva to nymph, or from nymph to adult. Developmental phases of the parasite in the spleen and other internal organs have been observed by Koch and others. Their significance in the cycle of development is not yet known.

Babesia canis.—A piroplasmosis of the dog has been observed in France, Italy, South Africa and some other places. Possibly the European and African parasites are different. An Indian canine piroplasmosis is certainly due to a distinct species. The infection, which is termed "malignant jaundice" or "bilious fever," is accompanied in its acute form by anemia, hemoglobinuria, and usually some jaundice. The parasite is intracorpuscular and is very similar to the parasite of Texas fever. Only dogs are susceptible to this disease, all other animals proving refractory. As is the case with other piroplasmoses, the disease in the dog is conveyed by tick bite. The African variety of the disease cannot be conveyed by the larva, but only by adult ticks raised from infected eggs. In the European

form the larvæ or nymphs as well as the eggs may become infected directly from the dog. "The variations in regard to the mechanism of transmission, especially the time factor, indicate that obligatory changes in the life-history take place in the insect body" (Calkins).

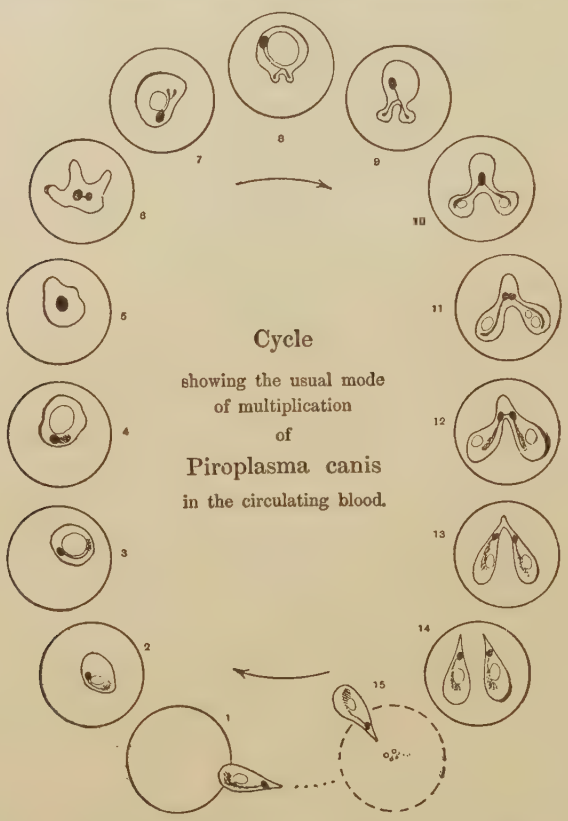


Fig. 164.—*Piroplasma canis:* (1) A free piriform parasite which has just left a blood-corpuscle enters a normal corpuscle and (2–4) assumes a rounded form, remaining quiescent for a time, after which it grows in size. It then becomes actively ameboid (5, 6), and again becomes rounded (7). Two symmetrical processes (8–10) are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes (11–13) gives rise to a mature piriform parasite, which remains attached to its fellow by a thin strand of protoplasm. The parasites next become separated (14) and, by active swimming movements, burst out of the corpuscle (15) whose hemoglobin escapes into the plasma. The free parasite immediately re-enters a fresh corpuscle (George H. F. Nuttall, in Johns Hopkins Hospital Bulletin).

The nature of these changes has not yet been made out. Probably a sexual cycle occurs in the tick. The serum of recovered animals renders virulent blood innocuous, and the same is true, although in

lesser degree, of the serum of naturally immune animals like the sheep.

Nuttall and Graham Smith[1] have made an exhaustive laboratory study of this disease and have described with great wealth of detail that part of the life cycle of *Babesia canis* which is passed in the blood of the dog. According to these investigators, the development takes place in the following manner (Figs. 164 and 165):

A free pyriform parasite enters a normal red blood-corpuscle and rapidly assumes a rounded form. It then enlarges and passes

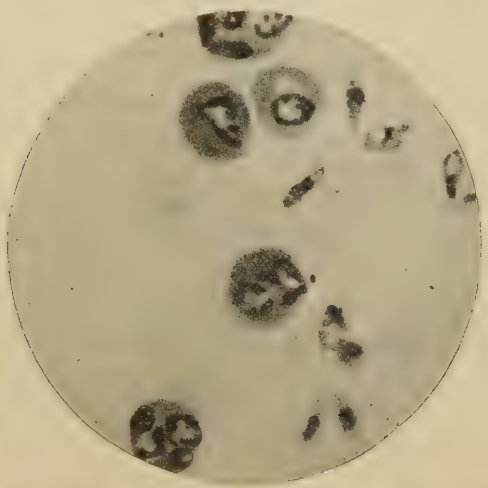


Fig. 165.—*Babesia canis* in blood of a dog (Nuttall and Graham Smith).

through an actively ameboid stage, at the end of which it again becomes rounded. After a short period of quiescence in this condition it protrudes two symmetrical processes, which rapidly grow and become pear-shaped. The protoplasm of the parasite flows into these processes, and its body consequently gradually diminishes until it is represented by a minute rounded mass to which the pyriform processes are attached. Eventually this also disappears, and finally two mature pyriform parasites are left, which are joined together for a time by a thin strand of protoplasm. After a variable time these parasites are liberated by the rupture of the cor-

[1] Nuttall and Smith: Jour. of Hygiene, 1904, 4, p. 219; 1905, 5, pp. 237, 250; 1906, 6, p. 586; 1907, 7, p. 232. See also Kinoshita: Arch. Protistk., 1907, 8, p. 294; Christophers: Sci. Memoirs, Med. and Sanit. Dept., Gov't of India, No. 29, 1907; Breinl and Hindl: Ann. Trop. Med., 1908, 2, p. 233.

puscles, and swim away to enter fresh corpuscles and repeat the process.

Kleine[1] and Miyajima[2] report successful cultivation experiments with this parasite (Kleine) and with *Pir. parvum* (Miyajima). Growth with developmental changes is said to have occurred in dog's blood and in blood broth.

Nuttallia equi.—A disease of horses especially studied by Theiler[3] in South Africa, and presenting many striking resemblances to the piroplasmosis of dogs, is apparently caused by an organism specific for the horse. The parasites appear much smaller than the true Babesia. Inoculation experiments from one horse to another are sometimes successful, but by no means invariably so. Laboratory experiments with ticks (*Rhipicephalus evertri*) have given positive results, and this species is probably responsible for communicating the disease in Africa.

Piroplasma ovis.—A piroplasmosis of the sheep was first discovered in 1888 by Babes,[4] although this investigator did not recognize the protozoan nature of the parasite. The disease is transmitted by a tick (*Rhipicephalus bursa*). Especially interesting is the fact that the larvæ and nymphæ raised from infected eggs are incapable of conveying the infection, although the mature ticks of the same brood are infective. In this respect the sheep piroplasmosis resembles that of the dog and differs from Texas fever.

Other Pathogenic Protozoa

Among other pathogenic sporozoa may be mentioned those sporozoa which are generally termed coccidia. One of these, *Eimeria stiedæ*, is a very common parasite in rabbits, where it is found especially in the liver. In these animals it evokes cirrhotic changes and other chronic inflammatory processes. Young animals are very susceptible and often die in large numbers. There is at least one species of coccidium infecting man, *Isospora hominis*, and Wenyon[5] believes that there are two. Three species of Eimeria that have been found in human feces are now known to be the

[1] Kleine: Ztschr. f. Hyg., 1906, 54, p. 10.

[2] Miyajima: Philippine Jour. Sci., 1907, 2, p. 83.

[3] Theiler: Schweizer Archiv. f. Tierheilk., 1901, 43, p. 253, trans., Jour. Comp. Path. and Ther., 1902, 15, p. 40.

[4] Babes: Compt. rend. Acad. Sci., 1892, 115, p. 359.

[5] Wenyon: Ann. Trop. Med. and Parasit., 1923, 17, 231–288.

oöcysts of fish coccidia which have passed unchanged through the digestive tract.[1] Although various digestive disturbances have been ascribed to Isospora in man, they may be due to other causes.

Several of the myxosporidia, a subdivision of the *Cnidosporidia*, are definitely associated with the production of disease in fish, one of the best known of these cases being the pox disease of carp caused by *Myxobolus cyprini*. The parasite causing the disease of silkworms known as pébrine, celebrated by Pasteur's classic

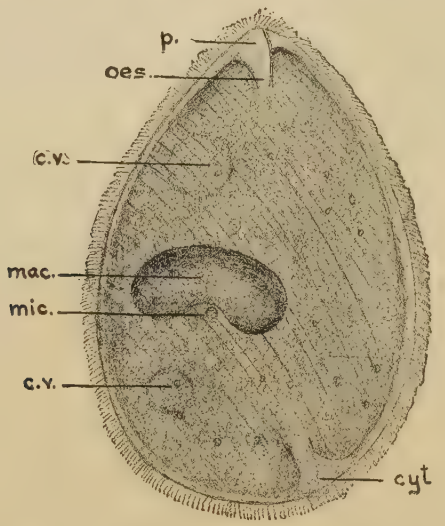


Fig. 166.—*Balantidium coli* from man. c.v., contractile vacuole; cyt., cytopyge; mac., macronucleus; mic., micronucleus; oes., oesophagus; p., peristome. (From Hegner in Hegner and Taliaferro's "Human Protozoölogy," courtesy of The Macmillan Company.)

researches, also belongs to this group (*Nosema bombycis*). Another group which is generally placed in the *Cnidosporidia*, the sarcosporidia, contains a number of parasites, most of which are characterized by their ability to invade muscle-fibers. A large variety of animals are subject to invasions by parasites of this class, and there are several instances of sarcosporidiosis reported in man.[2]

Some investigators recognize three species of the Ciliophora as parasitic in man: *Balantidium coli*, *Bal. minutum* and *Nyctotherus faba*. The parasitism of the last two is very doubtful. *Balantidium coli* (Fig. 166) is a typical ciliate and occurs in the pig and

[1] Thomson and Robertson: Brit. Med. Jour., 1926, 1, pp. 282, 420.

[2] Hegner and Taliaferro: "Human Protozoölogy," New York, 1924, p. 373.

monkey as well as in man. It is an inhabitant of the large intestine and sets up the same type of lesion as *E. histolytica*. In fact, the pathology of the diseases and the life cycle of the two parasites are very similar. Some persons with *Bal. coli* infection suffer from acute dysentery, but probably the majority are carriers without symptoms. Just as with *E. histolytica*, it is these carriers who disseminate the infection. *Bal. coli* is widely distributed over the earth, although the exact incidence has been very little studied.

Barret and Yarborough[1] have successfully cultivated *Bal. coli* for over thirty-two days, involving eleven subcultures, in a 1:17 dilution of inactivated human serum in 0.5 per cent. saline. The cultures were kept at 37° C.

[1] Barret and Yarborough: Amer. Jour. Trop. Med., 1921, 1, p. 161.

CHAPTER XXXII

THE FILTERABLE VIRUSES[1]

It was first shown by Löffler and Frosch[2] that there are micro-organisms pathogenic for man so small or so plastic as to be able to pass through the pores of porcelain or infusorial earth filters. These observers while studying a highly contagious disease of cattle, transmissible also to man (foot-and-mouth disease), attempted to obtain germ-free lymph from infected animals by the usual method of filtration, but found to their surprise that 0.02 c.c. of the filtered lymph was still able to produce infection. From an animal infected with the filtered lymph they transferred the infection through a series of six animals. It was calculated that the last one in the series received less than one two-billionth part of the original lymph. Since one fifty-thousandth part of the original lymph was not infectious, there was no escape from the conclusion that the virus passing the filter must be a living organism capable of multiplication.

As shown by Helmholtz, the nature of light rays restricts the limits of clear vision to objects of a size not less than 0.1 to 0.2 μ, and it was at first supposed that the filter-passers were beyond these limits of vision, were, in short, "ultramicroscopic," but it has been found that at least some of the filterable viruses—for example the micro-organism that causes the pleuropneumonia of cattle—are visible with high powers of the microscope. In many cases, however, examination of the infectious filtrate by the ordinary methods of illumination shows the presence of nothing recognizable as a micro-organism. It was at one time thought that the method of dark field illumination could be profitably employed in the study of such filtrates. This method consists in intense illumination of the object, so that the object becomes visible by diffracted light. The phenomenon is the same as that which is observed when a ray of sunlight passes into a darkened room. The exceedingly small

[1] An excellent review has been given by Rivers: Jour. Bact., 1927, 13, p. 16; see also Rivers: "Filterable Viruses," Baltimore, 1927.

[2] Löffler and Frosch: Centralbl. f. Bakt., Orig., 1898, 23, p. 371.

particles or motes of dust are made perceptible by the diffracted light, while with ordinary illumination (transmitted light) they are quite invisible. The use of sunlight in the ultramicroscope permits objects as small as 0.004 μ to become visible. While the ultramicroscope has been of value in the investigation of colloidal solutions and in the study of such tenuous organisms as the spirochetes of syphilis and yellow fever, it has added little or nothing to our knowledge of most of the filterable viruses. One reason for this is that such a method of illumination makes all the small particles in suspension appear as luminous points without differentiation as to size, shape, or structure. It is hence impossible to distinguish any minute living organism that may be present from the multitude of other particles in organic fluids, which likewise become visible by this method.

Since the number of demonstrated filter-passers is now very large, numbering probably about 50, it is always advisable in investigating an infectious disease of unknown etiology to determine whether or not the filtrate from infectious blood or lymph or organ emulsion is itself infectious.

Many precautions are necessary in carrying out the filtration process in order to demonstrate surely the existence of a filterable virus. Some of these are as follows: The filtration must be completed within as short a time as possible—always within two hours—in order to avoid the possibility that the micro-organisms may grow through the pores of the filter rather than pass through. Many of the familiar pathogenic bacteria will grow through a filter if the process of filtration is long-continued. For the same reason it is not advisable to filter at incubator temperature. The pressure—positive or negative—used in filtration should not exceed 500 mm. of mercury. Pressures amounting to several atmospheres are inadmissible. In order to lessen the protein content of the fluid to be filtered, dilution with sterile water or salt solution, 1:40 to 1:100, is desirable. The integrity of the filter and the freedom of the filtrate from ordinary bacteria should always be determined. Organisms like Bacillus pyocyaneus are sometimes added as test-objects to the fluid to be filtered.

It has been shown by Kramer[1] that the nature of the filtering material is important and that filters that have a positive electrical

[1] Kramer: Jour. Infect. Dis., 1927, 40, p. 343.

charge (calcium carbonate, magnesium oxide) remove viruses that are able to pass through the usual filters that have a negative electrical charge (siliceous material).

From the work already done on "the filterable viruses" it is clear that they do not constitute a homogenous group. Organisms of very diverse biological character appear to possess the ability in common of passing through certain kinds of filters under certain conditions. Size is not the only determining factor. Some of the filterable viruses appear to be very small bacteria, others small protozoa, while still others of quite unknown nature may lie beyond the limits of vision. The existence of a minute filterable stage in the life-history of a relatively large and structurally complex microorganism is a possibility that must be reckoned with. Some of the diseases caused by filter-passing viruses are conveyed by insects, others are transmitted by contact, by respiratory infection or by direct inoculation (rabies). Some of these can be cultivated from generation to generation in artificial culture media, others seem to multiply only in the presence of living tissue cells. No "wild" filterable viruses free in nature have been found.

One of the most remarkable and characteristic features of a number of the diseases with which typical filterable viruses are associated is the presence of cell-inclusions. These singular bodies may be located either in the cytoplasm or the nucleus or in both. In some diseases the cell-inclusions have a diagnostic value, as is the case with the Negri bodies in rabies. It is not known whether the cell inclusions are products of degeneration or whether they represent some phase of the virus itself; they may not have a uniform significance. Attempts to produce them artificially have not been successful.

Some investigators have been led to believe that certain of the so-called filterable viruses are not living organisms at all but active enzyme-like chemical substances. Studies on the bacteriophage (p. 116) have been thought to support this view. It is not certain, however, that the bacteriophage itself is inanimate. The virus of the mosaic disease of tobacco has many remarkable qualities and has been supposed by Beijerinck to be a "contagium vivum fluidum," that is, unformed living material capable of propagating itself; there is evidence, however, that this virus is a living microbic body.[1]

[1] Olitsky: Jour. Exper. Med., 1925, 41, p. 129.

Smallpox.—Although vaccination against smallpox has long been successfully practised, no specific micro-organism has been isolated in pure culture and shown experimentally to form the basis for this procedure. Bacteria of various kinds, especially streptococci, are often found in the pustules, but it is generally believed that they are accidentally present or are secondary invaders; there is no evidence that any particular bacterial species is the primary exciting cause. The virus of smallpox and vaccinia can pass through a Chamberland filter. The epithelial cells of smallpox lesions contain peculiar bodies, first regarded by Guanieri in 1892 as parasitic protozoa, and later subjected by Councilman, Calkins, and their

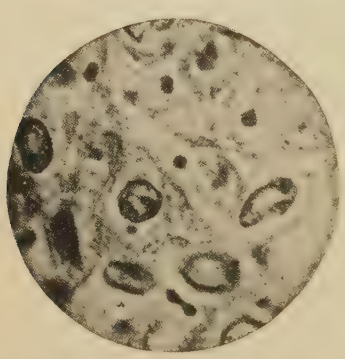


Fig. 167.—Advancing stage of smallpox vesicle; young cytoplasmic forms of parasites in the epithelial cells. × 200 (Councilman, Macgrath, and Brinckerhoff).

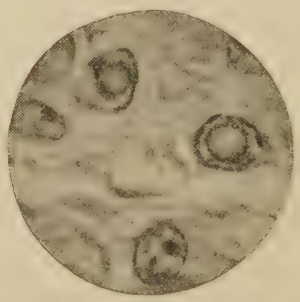


Fig. 168.—Intranuclear form of smallpox parasite (Councilman, Macgrath, and Brinckerhoff)

co-workers to thorough examination.[1] These later investigators regard the intracellular bodies as undoubted parasites which pass through a definite cycle in the cytoplasm of the epithelial cell (Fig. 167). Similar bodies are found in the skin lesions due to vaccination. There is this important difference, however, between the histologic appearances in vaccine lesions and those in smallpox. The affected cells in smallpox show definite nuclear changes which are interpreted as inclusions and looked upon as representing an intranuclear phase (sexual cycle?) (Fig. 168) of the parasite. In the lesions of vaccination as they appear in man, the monkey, and other animals no intranuclear bodies are found. Both the cytoplasmic and nuclear bodies in smallpox lesions show progressive changes which

[1] Councilman *et al.*: Jour. Med. Res., 1904, 11, pp. 1–361.

are regarded as developmental stages in the life of the parasite. The parasitic nature of these bodies has not, however, gone unchallenged. Ewing[1] has brought forward evidence indicating that the changes observed in the cell are forms of cell degeneration due to some kind of toxin, and are not definite parasites.

A form of smallpox (variola inoculata) which is less serious in character than true smallpox has been produced in man by direct inoculation with the contents of smallpox vesicles. At one time this method of inoculation was practised to some extent in England and some other countries as a valuable means of prophylaxis, the mild attacks of the disease so engendered imparting a high degree of protection against the naturally contracted virulent type. Smallpox has been experimentally produced in monkeys by cutaneous inoculation, and manifests the same characters as inoculation-smallpox in man.

The relation between smallpox and the disease cowpox or vaccinia—a name given to a certain vesicular eruption on the udder of cows—was clearly established by the celebrated observations of Jenner in 1798. Noting that dairy workers who had had cowpox were less liable than others to contract smallpox, Jenner advocated on this and other substantial grounds the systematic inoculation of cowpox virus as a protection against smallpox. The practical success achieved by the method of vaccination is matter of common knowledge. No one with any understanding of the nature and force of scientific evidence questions that by this means smallpox is today held in check.

It is now generally believed that the cowpox of cattle is a modified form of smallpox; indeed, typical cowpox has been produced in heifers and in rabbits by inoculation with human smallpox virus. The method of vaccination is therefore a process of active immunization with the living micro-organism of smallpox attenuated by passage through the body of the cow. Vaccine is neutralized by the serum of immunized animals, which evidently contains some substance capable of exerting a germicidal or inhibitory action upon the specific agent. Injection of immune serum seems to be without effect upon the course of a case of smallpox.

Many observers believe that the secondary infection of smallpox lesions with streptococci, which almost always, if not invariably,

[1] Ewing: Jour. Med. Res., 1904, 12, p. 509; 1905, 13, p. 233.

occurs, is more directly responsible for bringing about a fatal termination than the effects wrought by the specific parasite.

The disease called *alastrim* which is widely prevalent in Brazil and the West Indies is believed by many to be a mild form of smallpox. Others regard it as an independent, closely related disease. Vaccination against smallpox affords a powerful protection against alastrim.

Rabies or Hydrophobia.—All mammalia are susceptible to rabic virus. Man is infected most frequently by the bite of the dog, but may also contract the disease through the bites of cats, wolves, cattle, and other animals. The bite of wolves is much more virulent than that of dogs. Bites upon the hands and face and other parts having a rich nerve-supply are most apt to result fatally. If the part bitten is covered by clothing or hair, less virus will enter the wound than if the bite is made on an exposed surface, and such bites are correspondingly less dangerous. The most reliable statistics indicate that about 16 per cent. of persons bitten by rabid dogs become infected. The virus is contained in the saliva of infected subjects, including man, and, according to Nocard and Roux, is always present in the saliva of the dog twenty-four to forty-eight hours before the animal shows any sign of illness. The virus is also always present in every part of the central nervous system, especially in the medulla. Experimentally it has been found that the virus makes its way from the point of inoculation to the central nervous system chiefly by way of the nerve-trunks. In this respect and some others, rabies presents a close analogy to tetanus. The virus is readily destroyed by heat and drying and is rendered inert by direct sunlight in about forty hours.

No particularly characteristic and constant changes were found in the tissues of animals infected with rabies until Negri,[1] in 1903, described certain peculiar bodies as occurring in the large nerve-cells of the central nervous system. Subsequent investigators have confirmed Negri's observations, and the "Negri bodies" are now regarded as specific to hydrophobia (Fig. 169). The occurrence of these bodies, moreover, has been shown to be very important in making possible a rapid histologic diagnosis. The examination for Negri bodies may be made directly from the fresh tissues by

[1] Negri: Boll. d. Soc. med.-chir. di Pavia, 1903, p. 88; Ztschr. f. Hyg., 1903, 43, p. 507.

means of the smear method. The method is as follows: After removing the top and occipital portions of the skull, leaving the brain in position, cut out small pieces (3 to 4 mm.) of the gray substance from—(*a*) the cerebral cortex in the region of the crucial sulcus, (*b*) the cortex of the cerebellum, and (*c*) the hippocampus major (Ammon's horn). Place a piece of the tissue on a well-cleaned slide, crush it with a cover-slip, and draw it slowly and evenly toward the end of the slide, leaving a thin smear of well-spread nerve-cells. The smears are then dried in the air and may be stained by various methods. Williams and Lowden[1] have suggested the following modification of the Giemsa stain (p. 57) made by van Gieson for quick differentiation: "To 10 drops of distilled water three drops of a saturated alcoholic solution of rose anilin violet and six drops of Löffler's solution of methylene-blue are added. The smears are fixed while moist in methyl-alcohol for one minute. The stain is then poured on, warmed till it steams, poured off, and the smear is rinsed in water and allowed to dry."

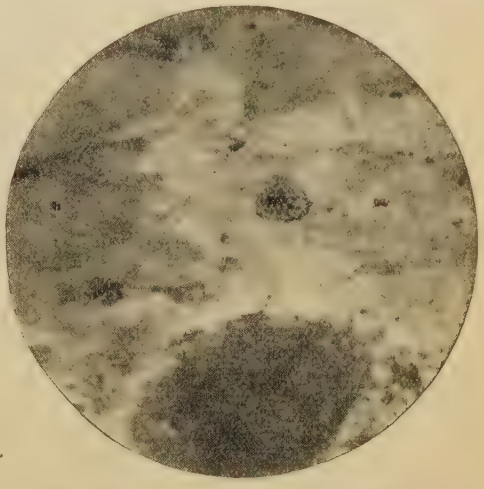


Fig. 169.—A rounded "Negri body" showing well the complete circle of chromatoid granules about the central body. From street rabies. × 2000 (Williams and Lowden).

Negri himself has from the first maintained that these bodies are protozoan parasites, and his opinion has been shared by a number of other workers. Williams and Lowden[1] give the following reasons for concluding that the Negri bodies are organisms belonging to the class Protozoa: "(*a*) They have a definite, characteristic morphology; (*b*) this morphology is constantly cyclic, *i. e.*, certain forms always predominate in certain stages of the disease, and a definite series of forms indicating growth and multiplication can

[1] Williams and Lowden: Jour. Infect. Dis., 1906, 3, p. 452.

be demonstrated; (*c*) the structure and staining qualities, as shown especially by the smear method of examination, resemble that of certain known protozoa, notably of those belonging to the suborder Microsporidia."

The Pasteur method of treatment for hydrophobia is based upon the fact that the rabic virus in the spinal cord of rabbits loses strength at a fairly regular and even rate when the cord is removed from the body after death and carefully dried. In the preparation of material for the preventive treatment, rabbits are inoculated with "fixed virus"—a term given by Pasteur to virus that is so exalted in virulence by successive passages through rabbits that it will produce the death of these animals in six or seven days. Beyond this point no increase of virulence can be obtained; hence the name, fixed virus. The spinal cord is removed aseptically from rabbits killed by the inoculation of fixed virus, cut into three pieces, and suspended over a solution of caustic potash in a drying chamber. Here the cords are kept in the dark and at a constant temperature of 23° C. for fourteen days. Emulsions of the dried cord are prepared in sterile salt solution or broth and injected every day, or sometimes more frequently, during a period of fifteen to twenty-one days, the interspacing of doses and duration of treatment being determined by the nature of the case. As a rule, the most attenuated material (fourteen-day cord) is injected first, and this is followed by virus of gradually increasing strength. Over 30,000 individuals bitten by rabid animals have been treated at the Pasteur Institute in Paris, with a mortality of less than 1 per cent., figures that probably mean the saving of the lives of between two thousand and three thousand persons. The method is essentially one of active immunization, and involves a race between the action of the attenuated virus and that of the virulent virus introduced by the bite of a rabid animal. It follows that the preventive treatment should always be begun at the earliest possible moment after the bite. In a certain proportion of cases a spinal cord lesion, "treatment paralysis," seems to follow the treatment. The pathogenesis of this condition is unknown. A fatal result is rare.[1]

The serum of animals immunized against hydrophobia possesses considerable protective power, and according to some investigators, has also a marked curative effect. Favorable results have been

[1] W. A. Jones: Jour. Amer. Med. Assoc., 1909, 53, p. 1626.

reported from the use of immune serum, especially in cases of severe bites about the head, or in persons who have delayed beginning the Pasteur treatment.[1]

Since nearly all cases of hydrophobia are due to the bite of rabid dogs, it is of the utmost importance to check the spread of the disease in these animals. This can be most effectively and humanely accomplished by the strict enforcement of proper muzzling regulations. The experience of Great Britain is worth citing.

Year	Cases of Rabies
1887	217
1888	160
1889	312
Muzzling enforced:	
1890	129
1891	79
1892	38
Opposition to muzzling; ordinance relaxed:	
1893	93
1894	248
1895	672
Muzzling again enforced:	
1896	438
1897	151
1898	17
1899	9
1900	6
1901	1
1902	13
1903–07	0

Measles.—At the present time measles is one of the most serious diseases of early childhood. In 1924 the deaths reported from measles in the registration area of the United States numbered 8517, a figure larger than the deaths from scarlet fever (3122) or from typhoid (6677) and but slightly lower than the deaths from diphtheria (9316).

Hektoen[2] was first to obtain rather convincing evidence that the specific agent of measles is contained in the blood. Blood drawn from the veins of a patient and mixed with ascites broth was found to give no visible growth when incubated at 37° C. for twenty-four hours. A few cubic centimeters of this mixture injected into a healthy man produced the typical symptoms and eruption after

[1] Tizzoni and Centanni: (Abs.) Centralbl. f. Bakt., 1895, 18, p. 246.

[2] Hektoen: Jour. Infect. Dis., 1905, 2, p. 238.

the usual period of incubation. Sellards,[1] however, in carefully controlled observations upon 8 subjects failed to transmit measles by the injection of blood.

Anderson and Goldberger[2] seemingly succeeded in communicating the disease to the lower animals (monkeys) in definite and regular fashion. The apparent reason for the preponderating negative and irregular results of earlier experimenters is the fact that human blood is infective for monkeys during a very limited period. This period begins before the appearance of the characteristic eruption, and continues for about twenty-four hours after the eruption shows itself. "At the end of about twenty-four hours from the first appearance of the eruption the infectivity of the blood for the rhesus monkey already appears very greatly reduced, and becomes progressively less thereafter."

Further experiments by the same investigators[3] demonstrated the presence of the virus of measles in the mixed buccal and nasal secretions, and showed that the virus could pass through a Berkefeld filter, could resist desiccation for twenty-five and a half hours and freezing for twenty-five hours, while infectivity of the virus was destroyed by heating for fifteen minutes at 55° C.

In 1917 Ruth Tunnicliff reported the isolation of a green-producing coccus from the blood in the pre-eruptive and eruptive stages of measles.[4] The relations of this organism to measles were carefully studied by Tunnicliff, and the results reported in a series of papers.[5] At first the coccus could be cultivated only anaërobically but in the second generation it grew aërobically. It is gram-positive, small and grows in pairs (diplococcus) and short chains. Nothing distinctive has been reported concerning its fermentive powers and other cultural characteristics. The organism was grown from the blood, sputum, nasopharyngeal secretions and ear discharges of measles patients in the pre-eruptive stage and in the earlier stages of rash. Rabbits inoculated with the Tunnicliff coccus manifested symptoms and lesions showing some resemblance

[1] A. W. Sellards: Bull. Johns Hopkins Hosp., 1919, 30, p. 257.

[2] Anderson and Goldberger: Public Health Reports, Washington, 1911, 26, pp. 847, 887.

[3] Goldberger and Anderson: Jour. Amer. Med. Assoc., 1911, 57, pp. 476, 971.

[4] Tunnicliff: Jour. Am. Med. Assoc., 1917, 68, p. 1028.

[5] See for example: Jour. Infec. Dis., 1918, 22, p. 462; 1918, 23, p. 572; 1919, 24, p. 76; Ibid., p. 181; 1922, 31, p. 382; 1925, 37, p. 193.

to measles. Skin tests with killed cultures of the coccus gave positive reactions in 26 persons who had not had measles and in only 1 out of 29 persons with a history of measles.

In 1926 Ferry and Fisher reported the demonstration of an extracellular toxin in filtered culture of green-producing streptococci from measles; with this toxin they obtained positive skin reactions in a considerable proportion of susceptible persons.[1] Ferry and Fisher believed that the organism isolated by them differed from that described by Tunnicliff in growing aërobically and in producing a specific soluble toxin. Variations in cultural methods may explain the discrepancies. Tunnicliff and Taylor[2] later obtained from Tunnicliff's diplococcus an extracellular toxin which gave a specific skin reaction in persons with a negative history of measles in about the same percentage as that obtained by Ferry and Fisher. The serum from goats immunized with the Tunnicliff diplococcus seems to confer a high degree of protection when given to measles contacts not later than the fifth day of exposure.[3] Many bacteriologists believe that the green-producing diplococcus of Tunnicliff and the green-producing streptococcus of Ferry are identical.

A gram-negative anaërobic diplococcus, isolated by the Italian bacteriologist Caronia, differs somewhat according to the published description, but perhaps may be the same as the organism studied by Tunnicliff and Ferry.

Long and Cornwell[4] were unable to isolate any toxin-producing green streptococci from the blood of 26 measles patients.

There is no doubt that the blood of persons who have recovered from measles contains substances of immunizing power. The whole blood, the blood plasma, and the serum from measles convalescents have been used quite extensively for prophylactic purposes in various parts of the world.[5] In New York City where about 1000 exposed children, mostly in institutions, were injected with convalescent serum measles was either prevented altogether or modified so that the attack was very mild.[6]

[1] Ferry and Fisher: Jour. Amer. Med. Assoc., 1926, 86, p. 932.

[2] Tunnicliff and Taylor: Jour. Amer Med. Assoc., 1926, 87, p. 846.

[3] Tunnicliff and Hoyne: Jour. Infect. Dis., 1926, 38, p. 48; Hoyne and Gasul: Jour. Amer. Med. Assoc., 1926, 87, p. 1185.

[4] Long and Cornwell: Jour. Infect. Dis., 1927, 40, p. 408.

[5] Cf. Zingher: Jour. Amer. Med. Assoc., 1924, 82, p. 1180.

[6] Park and Freeman: Jour. Amer. Med. Assoc., 1926, 87, p. 556.

Foot-and-mouth Disease.—A disease of cattle and other domestic animals, characterized by fever, digestive disturbances, and especially by the appearance of a vesicular eruption on the mucous membrane of the mouth and on the skin of the hoof or between the toes, has long been known in parts of Europe. On several occasions it has been imported into the United States, a very extensive outbreak occurring in 1914, when over 2000 herds and 100,000 cattle were affected. The stamping out of the disease was brought about by the slaughter of infected and exposed animals and by the application of rigorous methods of quarantine and disinfection. The disease may be communicated to man by milk or milk-products or by contact with infected animals. Among cattle the mortality is apt to be high, especially in young animals. In man the disease is usually of a mild type, but is sometimes fatal in children.

The virus is present in the contents of the vesicles and ulcers and also in the milk, saliva, urine, and feces. Animals that have had the disease may carry the virus for several months. The period of incubation is usually short, two to seven days, but may be much longer. The infection may be carried by fodder, milk, or by the hands or clothing of drovers. It may also be conveyed in cattle cars, stalls, and feeding and drinking troughs.

No specific micro-organism has been found in the disease. It has been demonstrated, however, by Löffler and Frosch[1] that the disease can be produced by the inoculation of lymph from the vesicles after the lymph has been filtered through the finest Berkefeld filters. On this ground the cause of the disease has been classed as an "ultramicroscopic virus." The virus retains its virulence for months if kept cool and moist, but is rapidly destroyed by a temperature of 60° C. and by drying. A protective substance exists in the serum of animals convalescent from the disease, and in experimental work the principle of passive immunization can be successfully practised.

Epidemic Infantile Paralysis (Acute Poliomyelitis, Heine-Medin Disease).—This disease was recognized as a specific malady more than fifty years ago by Jacob von Heine. In more recent times attention became especially directed to it through the Swedish outbreaks of 1899 and 1905 (1000 cases), which were

[1] Löffler and Frosch: Centralbl. f. Bakt., I, 1898, 23, p. 371.

carefully investigated by Medin. The malady has since appeared in Germany and in many parts of the United States:[1] in 1910 approximately 900 cases were reported in the United States. Young children from one to two years old seem most liable to attack, adults being seldom affected. The mortality ranges from 6 to 15 per cent., and 75 per cent. or more of the survivors are permanently crippled. Flexner and Lewis[2] first succeeded by the method of intracranial inoculation in carrying the virus through a series of monkeys. In all the successive transfers lesions similar to those of human poliomyelitis were observed.

The Specific Micro-organism.—Flexner and Noguchi[3] were first to report cultivation of a micro-organism in epidemic poliomyelitis. The medium employed was human ascitic fluid to which is added a fragment of sterile fresh tissue (normal rabbit kidney). Growth was at first obtained only under anaërobic conditions. The surface of the medium may be covered with a deep layer of sterile paraffin oil, or anaërobic jars may be used. Fragments of infected central nervous system, preferably the brain, are best introduced directly into the culture-medium, but emulsions or filtrates of the nervous system may also be employed. After about five days' incubation at 37° C. a faint opalescence appears about the fragments of tissue at the bottom of the tube.

The supposed micro-organisms are globoid bodies, measuring from 0.15 to 0.3 μ in diameter, devoid of independent motility, and grouped in pairs, chains, and masses according to the conditions of growth and multiplication. Gram's stain is retained with more or less intensity, depending on the age of the culture and the constitution of the culture-medium.

Whether the globoid bodies so described represent the specific causal agent of poliomyelitis is still uncertain.

Micrococci in Poliomyelitis.—Several investigators[4] have reported the cultivation of streptococci from the throat, blood, and cerebrospinal fluid of clinical cases of poliomyelitis. Two views are held as to the significance of these cocci, one group of workers hold-

[1] Lovett and Richardson: Amer. Jour. Dis. Children, 1911, 2, p. 389.

[2] Flexner and Lewis: Jour. Amer. Med. Assoc., 1909, 53, p. 1639.

[3] Flexner and Noguchi: Jour. Exper. Med., 1913, 18, p. 460.

[4] G. Mathers: Jour. Amer. Med. Assoc., 1916, 67, p. 1019; Rosenow, Towne, and Wheeler: Ibid., 1916, 67, p. 1202; Nuzum and Herzog: Ibid., 1916, 67, p. 1205.

ing that the streptococci are causally related to poliomyelitis and that they are a phase in the life-history of the globoid bodies just described, the other that they are secondary invaders only. The latter view is maintained on the ground of the bacteriological findings,[1] the pathologic effects of the streptococci isolated[2] and therapeutic experiments with serum prepared from the streptococci.[3] On the other hand, the streptococcus appears to be present with a high degree of frequency in poliomyelitis monkey virus, to be strongly resistant to glycerol, to pass the filters commonly used in the study of poliomyelitis virus, and to produce in monkeys after three to four culture generations "conditions absolutely indistinguishable, clinically and anatomically, from the classical induced poliomyelitis in this animal." The streptococcus, however, grows aërobically on blood-agar in much the same way as a streptococcus of the Streptococcus viridans type, while the globoid organism of Flexner and Noguchi is not known to grow under aërobic conditions at all. It does not seem possible at present to state definitely the exact relationship of the streptococcus to the globoid bodies or its significance in poliomyelitis.

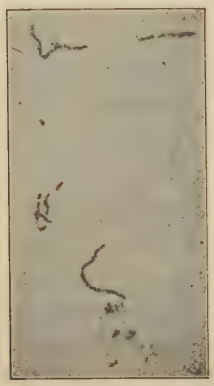


Fig. 170.—The microorganism of epidemic poliomyelitis. Chains and pairs of globoid bodies; × 1000 (Flexner and Noguchi).

Mode of Transmission.—The usual manner of transmission of epidemic poliomyelitis cannot be regarded as definitely determined. The fact that the virus is present in the secretions from the mouth and nose of children suffering from poliomyelitis has induced some investigators to believe that the infection is transmitted directly from person to person by means of contact. Many cases of the disease, however, have been observed under conditions where no connection whatever with a previous case could be traced. The existence of healthy carriers of poliomyelitis virus may perhaps explain the occurrence of such cases. The virus has been demonstrated in the mucous membrane of a monkey five and one-half

[1] Kolmer, Brown, and Freese: Jour. Exper. Med., 1917, 25, p. 789.
[2] Bull: Jour. Exper. Med., 1917, 25, p. 557.
[3] Amoss and Eberson: Jour. Exper. Med., 1918, 27, p. 309.

months after recovery from the experimental disease, and Lucas and Osgood[1] have found the virus in the nasal secretion of a human carrier four months after the acute stage of a second attack of poliomyelitis.

Certain facts in the epidemiology of infantile paralysis have been thought to indicate the probability of insect transmission.[2] The disease prevails under rural rather than urban conditions, and apparently shows no tendency to spread in congested city districts where many diseases due to contact are excessively prevalent. The seasonal incidence of infantile paralysis, which in most localities is most marked during the summer months, is likewise considered to favor the hypothesis of insect transmission, since the season of greatest prevalence corresponds with the maximum abundance of insect life. Some experimental evidence has been gathered bearing directly on this point. It has been shown that house-flies contaminated with the virus may harbor it in living and infective condition for at least forty-eight hours. Rosenau[3] reported that through the agency of the biting stable-fly (Stomoxys calcitrans) he had been able to transmit to healthy monkeys a disease in all essential respects like poliomyelitis. These flies, which had first bitten monkeys experimentally infected with poliomyelitis, were thought to be able to transmit the infection to healthy animals. Later experiments by Anderson and Frost,[4] by Sawyer and Herms,[5] and by Francis[6] have given negative results and make it appear doubtful whether the fly is the usual agent in spreading the disease in nature.

The view that dust is the medium by which the virus is conveyed has been advanced by some writers, but there is no convincing experimental evidence in support of this view, and the epidemiology of the disease is not in accord with it.

The occurrence in certain of the lower animals, as horses and dogs, of paralytic affections with symptoms like those of poliomyelitis has led a number of observers to suspect that infantile paralysis might be communicated to man from these animals. The

[1] Lucas and Osgood: Jour. Amer. Med. Assoc., 1913, 60, p. 1611.
[2] Cf. C. T. Brues: The Scientific Monthly, 1923, p. 471.
[3] Rosenau: Jour. Amer. Med. Assoc., 1912, 59, p. 1314.
[4] Anderson and Frost: Pub. Health Rep., Washington, 1913, 28, p. 633.
[5] Sawyer and Herms: Jour. Amer. Med. Assoc., 1913, 61, p. 461.
[6] Francis: Jour. Infect. Dis., 1914, 15, p. 1.

striking resemblances between poliomyelitis and rabies have been pointed out in this connection. It has never been definitely shown, however, that any of the lower animals suffer naturally from a disease which is actually identical with human poliomyelitis.

Krause's observations point to the gastro-intestinal tract as the portal of entry of the infecting agent.[1] Guinea-pigs, mice, and pigeons fed with intestinal contents from typical cases of infantile paralysis gave negative results. Rabbits, on the other hand, can be fatally infected by feeding. Several outbreaks of poliomyelitis have been traced to the use of raw milk.[2] In the epidemic at Cortland, N. Y., 1925, the dairy cows were milked by an individual in the acute stage of the disease and the milk was not pasteurized. Eight widely separated cases occurred among the users of the supply.

Although direct intracranial inoculation gives the best results, infection may be produced in other ways. Some investigators have succeeded in producing the disease by rubbing the virus on the sound nasal membrane, others by causing inhalation of an emulsion of the virus. The incubation period in monkeys averages about eight or nine days, but may range from five to forty-six. The disease is more fatal in monkeys than in children.

There are still many perplexing problems which arise in connection with possible modes of transmission of this disease, but the weight of evidence at the present time favors the likelihood of spread through direct or indirect contact with the virus discharged in the secretions from mouth and nose. Healthy carriers very likely play an important part in dissemination. Measures of quarantine directed toward preventing the spread from actual cases should certainly not be relaxed nor should the possibility of insect transmission be ignored. The seasonal incidence of the disease remains to be explained. Possibly it may rest upon increased infant susceptibility in hot weather.

Pathogenicity.—The virus is contained not only in the brain and spinal cord, but also in the mucous membrane of the nasopharynx, in the salivary glands, and in some cases in the cerebrospinal fluid and the blood. Virulent material is not weakened by drying, freezing, or five months' suspension in glycerol. On the other hand,

[1] Krause: Deut. Med.Wchnschr., 1909, 35, p. 1822.

[2] *E. g.*, Dingman: N. Y. State Jour. Med. 1916; Knapp, Godfrey and Aycock: Jour. Amer. Med. Assoc., 1926, 87, p. 635.

the virus is killed by relatively low temperatures (45° to 50° C. for thirty minutes) and by weak disinfectants (1 : 500 solution of permanganate of potash).

Monkeys that recover from the disease are refractory to a second inoculation. The serum of such animals will destroy the virus. Such facts indicate that possibly a preventive serum may be produced in the course of further investigation.

Rosenau and Havens[1] have carried a monkey virus progressively through eight series of young rabbits. The lesions in the rabbit are not so distinctive as those in man and monkey.

Pappataci Fever.—This disease is prevalent in warm countries, especially in the Mediterranean region. It is a fever of short duration and has been called "three-day fever." Leukopenia is always present, a circumstance that favors differential diagnosis from incipient typhus. One attack confers immunity. The virus is filterable, is present in the blood, and can be transmitted by sand-flies (pappataci flies) that have fed on the blood of patients with the fever (Doerr[2]). The flies do not become infective until seven or more days have passed after they are fed on a fever case. Prophylaxis is difficult, since the flies pass readily through an ordinary mosquito net.

Dengue,[3] or "breakbone fever," is an infectious disease restricted to warm climates. About 30,000 cases occurred in Galveston during the summer and fall of 1922. It presents certain resemblances to yellow fever and is transmitted by the same species of mosquito, Aëdes ægypti.

Mosquitoes may be infected from dengue patients during the first three days of illness; the dengue virus must remain in the female Aëdes for a period of more than ten days before the insect becomes capable of transmitting it to human beings; infected mosquitoes remain infectious throughout life; the virus does not pass through the eggs to the next generation of mosquitoes.

The epidemiology of dengue presents a striking resemblance to that of yellow fever. Epidemics "occur only when there are simultaneously present cases of dengue fever, large numbers of

[1] Rosenau and Havens: Jour. Exper. Med., 1916, 23, p. 461.

[2] Doerr: Berl. klin. Wchnschr., 1908, 45, p. 1847.

[3] A valuable monograph on dengue, containing the results of original investigation in the Philippines and a full bibliography, has been written by Siler, Hall, and Hitchens, Manila, 1926.

Aëdes ægypti and large numbers of non-immune individuals."[1] The best method of prevention seems to be that based on mosquito-control measures.

The virus of dengue is filterable, but little more is known about it. The animals generally used for experiment, including rhesus monkeys, seem quite insusceptible.

While one attack of dengue confers a certain degree of immunity, the resistance so acquired is relatively slight. Second, third and even fourth attacks are known to occur. It was shown in the Philippines by experimental inoculation that a severe attack might be produced after an interval as short as 53 days.

Other Diseases.—Among other affections of man that are regarded with more or less certainty as due to filterable viruses are *molluscum contagiosum* (Juliusberg, 1905), *warts* and *trachoma* (Bertarelli, 1908).

A number of important diseases of domestic animals are also believed to be due to filter-passing microbes. Besides the *pleuro-pneumonia of cattle* (p. 359), may be named *African horse sickness*, probably transmitted by mosquitoes, *sheep-pox*, *hog cholera*, *cattle plague*, and *swamp fever of horses*. Certain diseases of guinea-pigs and fowls (*fowl pest* and *fowl diphtheria* or *epithelioma contagiosum*) have also been found due to filterable viruses. Especially interesting is the *chicken sarcoma* studied by Rous and his associates.[2] For the experimental production of this disease the virus must be brought into close contact with injured tissue cells. The sarcomata produced by direct inoculation of the filterable agent do not differ from those resulting from the growth of a bit of transplanted sarcomatous tissue. When the virus is attenuated by heat it gives rise to tumors that grow slowly and retrogress frequently.

Silkworms and other insects are subject to attacks by specific filterable viruses. One disease of plants, the *mosaic disease* of tobacco and other plants, has been shown to be carried by a filterable virus.[3]

[1] Siler, Hall and Hitchens: Jour. Amer. Med. Assoc., 1925, 84, p. 1163.

[2] Rous: Jour. Exper. Med., 1911, 13, p. 397; Jour. Amer. Med. Assoc., 1912, 58, p. 1938; Rous and Murphy: Jour. Exper. Med., 1913, 17, p. 219.

[3] Historical summaries of work on the filterable viruses with references may be found as follows: Löffler: Centralbl. f. Bakt., I, Ref., Beihefte, 1911, 50, p. 1; Wolbach: Jour. Med. Res., 1912, 22, p. 1; Lipschütz: Kolle and Wassermann, Handbuch, 1913, 8, p. 345; MacCallum: Medicine, 1926, 5, p. 59; Simon: Physiol. Rev. 1923, 3, p. 483.

CHAPTER XXXIII

THE RICKETTSIÆ

The name Rickettsiæ (da-Rocha-Lima, 1916) was given in honor of Howard Taylor Ricketts who first described organisms of a peculiar kind in Rocky Mountain spotted fever and typhus fever. These organisms are extremely minute, pleomorphic, gram-negative and seem to have their natural habitat in certain arthropods, such as ticks, lice and bedbugs. Two and perhaps three diseases of man are thus far known to be caused by members of this group.

Rocky Mountain Spotted Fever.—This remarkable disease is restricted, so far as known, to a few localities. It is best known and apparently most fatal in the Bitter Root Valley of Montana, but occurs also in the mountainous parts of the neighboring states. Wolbach has given the following definition: "An acute, specific endangeitis chiefly of the peripheral blood-vessels, transmitted by a tick, Dermacentor venustus, and characteried by onset with chill, continued fever, severe pains in bones and muscles, headache, and a macular eruption, becoming petechial, which appears first on wrists, ankles, and back, then over the whole surface of the body."

The first important study of the disease was made by Wilson and Chowning.[1] These authors attributed the disease to a protozoön (Piroplasma), but the microscopic findings on which they based this opinion have not been confirmed by subsequent observers. Wilson and Chowning also suggested that the bite of the woodtick might be the means of conveying the disease. Communication by tick-bites was later experimentally demonstrated by King[2] and by Ricketts.[3] Extended and fruitful investigations were carried out by Ricketts,[4] who showed that the disease can be communicated to monkeys and guinea-pigs by the intraperitoneal injection of defibrinated blood of typical cases, and also by the bite of ticks that have

[1] Wilson and Chowning: Jour. Amer. Med. Assoc., 1902, 39, p. 131; Jour. Infect. Dis., 1904, 1, p. 31.

[2] King: Public Health Reports, July 27, 1906, 21,[2] p. 863.

[3] Ricketts: Jour. Amer. Med. Assoc., 1906, 47, p. 358.

[4] Ricketts: Jour. Infect. Dis., 1907, 4, p. 141.

fed on spotted fever patients. Both male and female ticks can transmit the disease. The virus can be kept alive in the laboratory either by alternate inoculation of monkey and guinea-pig or by continuous passage through the guinea-pig. In the latter animal the maximum infectivity of the blood seems to be between the third and fifth day after the beginning of fever. The virus is not filterable. Ricketts and Gomez[1] have shown that an attack of spotted fever in the guinea-pig and monkey produces a high and lasting active immunity which is characterized by the presence of protective antibodies in the serum. Passive immunity may be conferred by the injection of blood or serum from immunized animals, or by a mixture of such serum with virus. The curative power of the serum is low. Noguchi has prepared an immune rabbit serum which, mixed with the spotted fever virus, confers complete immunity on guinea-pigs.[2]

Wolbach[3] describes an organism found in the lesions of the disease in man, monkey, rabbit and guinea-pig, and in infective ticks. Three definite morphological types are recognized: (1) An intracellular bacillary form, without chromatoid granules, relatively large and present in ticks only during the stage of initial multiplication; found only in the cytoplasm of the alimentary tract cells; (2) a relatively small bacillary form with chromatoid granules, probably the same form seen within nuclei in sections of ticks, and rarely in smooth muscle cells in the blood-vessels of mammals; (3) a relatively large lanceolate paired form present in ticks and in the blood and lesions in mammals. This lanceolate form is characterized by its chromatoid staining reaction and is believed by Wolbach to be the form in which the virus is passed between the tick and mammalian hosts. The other two forms described are thought to be multiplication stages, and can be demonstrated only occasionally and with difficulty in mammalian hosts. Attempts to cultivate these organisms on all ordinary culture media have not succeeded.

Wolbach and Schlesinger,[4] however, were able to keep the organism alive, probably with some multiplication, in tissue plasma

[1] Ricketts and Gomez: Jour. Infect. Dis., 1908, 5, p. 221.

[2] Noguchi: Jour. Exper. Med., 1923, 38, p. 605.

[3] Wolbach: Jour. Med. Res., 1919, 41, p. 1. This is a comprehensive monograph, 197 pages, 21 plates, and full bibliography. See also Jour. Am. Med. Ass'n, 1925, 84, p. 723.

[4] Wolbach and Schlesinger: Jour. Med. Res., 1923, 44, p. 231.

cultures made with infected guinea-pig tissues and normal guinea-pig plasma. The name *Dermacentroxenus rickettsi* has been proposed by Wolbach for this organism. The facts on which belief in a causal relation is based are: "1. The constant occurrence of a micro-organism of distinctive size and morphology in the lesions characteristic of the disease in man, monkey, rabbit, and guinea-pig. 2. The constant presence of an identical micro-organism exhibiting undoubted evidences of developmental phases in ticks of proved infectivity, and the absence of similar forms in proved non-infective ticks. 3. The ability to recognize this specific micro-organism in the tissues and eggs of infective ticks in the presence of bacteria occasionally present in abundance in ticks of the species concerned."[1]

Tsutsugamushi disease is a Japanese disease very similar to Rocky Mountain spotted fever both clinically and in its singular restriction to certain localities.[2] Studies by Kitashima and Miyajima[3] emphasize the resemblance. The virus, like that of spotted fever, is not filterable and is extremely susceptible to chemical and physical agents. Tsutsugamushi disease is transmitted by the bite of the larva of a mite, Leptus akamushi, one of the order Acarida (which includes the ticks) of the class Arachnida. The monkey gives a characteristic reaction on experimental inoculation, but not the guinea-pig. The field mouse is believed to be the natural mammalian host of the virus. No causal organism has been surely identified, but Sellards[4] has obtained in pure culture an organism closely resembling the known rickettsiæ. A valuable summary of the Japanese investigations has been given by Kawamura and is available in an English translation (Studies on Tsutsugamushi Disease, Cincinnati, 1926, pp. 229).

Typhus Fever

Typhus fever, a highly contagious disease with an incubation period of five to eighteen days, a high fever, and a characteristic rash, has long been known to occur under conditions of over-

[1] Wolbach: Jour. Med. Res., 1919, 41, p. 185.

[2] Ashburn, P. M., and Craig, C. F.: Philippine Jour. of Sci., Sec. B., Med. Sci., 1908, 2, 1.

[3] Kitashima, T., and Miyajima, M.: Kitasato Arch. of Expt. Med., 1918, 2, p. 91.

[4] Sellards: Am. Jour. Pub. Health, 1923, 13, p. 989.

crowding and filth. The malady was clearly described by physicians of the sixteenth century and undoubtedly existed much earlier. Some writers believe that the great pestilence of Athens (430 B. C.), so graphically reported by Thucydides, was typhus. The disease has derived many of its popular names, such as "camp fever," "jail fever," "hospital fever," from the conditions under which it is spread. In England prisoners brought into court were sometimes the means of communicating the disease to judges, lawyers, and spectators, a circumstance that gave to certain court sessions the name of the "black assizes." Up to about 1850 typhus seems frequently to have assumed the proportions of an epidemic; probably the poorer quarters of many European cities were never free from it. In recent times the disease has greatly diminished and in some localities has disappeared altogether under modern modes of life. The abnormal conditions of war times, however, which facilitate overcrowding and uncleanliness, have given opportunities for such great extensions of typhus as occurred in 1915 in Serbia, where it is estimated that 135,000 persons perished of the infection. At the present time Mexico is its main seat in North America.

In New York City a mild form of typhus was differentiated from clinically similar affections by Brill.[1] Although it was regarded for some time as a distinct clinical entity (Brill's disease), its identity with typhus was demonstrated by Anderson and Goldberger.[2] What appears to be the same mild form of typhus occurs endemically in the southeastern United States.[3]

Mode of Transmission.—Nicolle[4] succeeded in transferring the disease from one monkey to another by the bite of the infected body-louse (Pediculus vestimenti). This has been confirmed by the experiments of Ricketts and Wilder[5] and Anderson and Goldberger.[6] It is possible that the head-louse (P. capitis) also conveys typhus, but this is not established. Shipley has given the following summary of our knowledge of these insect parasites:[7]

[1] Brill: Am. Jour. Med. Sci., 1910, 139, p. 484.

[2] Anderson and Goldberger: Public Health Reports, 1912, 27, p. 149.

[3] Maxcy: Public Health Reports, 1926, 41, p. 1213.

[4] Nicolle: Compt. rend. Acad. Sci., July 12, Sept. 6, 1909.

[5] Ricketts and Wilder: Jour. Infect. Dis., 1911, 9, p. 9.

[6] Anderson and Goldberger: Public Health Reports, (Dec. 24) 1909, $24,^2$ p. 1941; 1910, $25,^1$ p. 177; (May 31) 1912, $27,^1$ p. 835.

[7] A. E. Shipley: "The Minor Horrors of War," London, 1915.

"Lice form a small group of insects known as the *Anoplura*, interesting to the entomologist because they are now entirely wingless, though it is believed that their ancestry were winged. They are all parasites on vertebrates . . . Like almost all animals lower than the mammals, the male of the body-louse is smaller and feebler than the female. The former attains a length of about 3 mm. and is about 1 mm. broad. The female is about 3.3 mm. long and about 1.4 mm. broad. It is rather bigger than the hair-louse, and its antennæ are slightly longer. It so far flatters its host as to imitate the color of the skin upon which it lives . . . The habitat of the body-louse is that side of the underclothing which is in contact with the body. The louse, which sucks the blood of its host at least twice a day, is, when feeding, always anchored to the inside of the underclothing of its host by the claws of one or more of its six legs. Free lice are rarely found on the skin in western Europeans . . . But the under side of a stripped shirt is often alive with them . . . Mr. Warburton summarizes the life-cycle of the insects, as indicated by his experiments, as follows:

"Incubation period: eight days to five weeks.

"From larva to imago: eleven days.

"Non-functional mature condition: four days.

"Adult life: male, three weeks; female, four weeks.

"But we must not forget that these figures are based upon laboratory experiments, and that under the normal conditions the rate may be accelerated. From Mr. Warburton's experience it is perfectly obvious that, unless regularly fed, body-lice very quickly die. Of all the verminous clothing sent to the Quick Laboratory, very little contained *live* vermin. The newly hatched larvæ perish in a day and a half unless they can obtain food.

"Lice occur chiefly on the body (*Pediculus vestimenti*) and head (*P. capitis*). They are small grayish-white insects. The female lays about sixty eggs during two weeks; the eggs hatch after nine to ten days. The lice are small at first; they undergo several moults and grow in size, sucking blood every few hours, and attain sexual maturity in about two weeks. The eggs will not develop unless maintained at a temperature of 22° C. or over—such as prevails in clothing worn on the human body, or in the hair of the head. This is why, *when clothing is worn continuously*, men

are more prone to become infested with lice derived from habitually unclean persons, their clothing, bedding, etc. *P. capitis* lives between the hairs in the head, and the eggs, called 'nits,' are attached to the hairs. *P. vestimenti* lives in the clothing, to which it usually remains attached when feeding on man; it lays its eggs in the clothing, and usually retreats into the seams and permanent folds therein."

Lice that have bitten typhus patients during the febrile stage of the attack contain large numbers of peculiar minute bodies, especially in the epithelial cells of the digestive tract. Guinea-pigs injected with the crushed bodies of lice containing these bodies develop the febrile reaction considered characteristic of typhus infection.

The mild form of typhus endemic in the southeastern United States seems to differ in its mode of transmission from that of Old World typhus.[1] Epidemiologists find no evidence of louse transmission. Maxcy's observations suggest that a reservoir of this disease exists in rats or mice and that accidental transmission to man may occur through the bite of some parasitic blood-sucking arthropod.

Prophylaxis.—Protection against typhus in a typhus ridden district consists mainly in avoidance of the infected vermin. This is often very difficult for those whose work as physicians or nurses brings them in close contact with typhus patients. Several investigators of the disease, including the talented Ricketts,[2] have laid down their lives as the result of infection contracted during their studies, and there have been a number of eminent victims among German physicians during the great war and among American and English physicians and nurses in Serbia.

Among the measures recommended for avoidance of infection are the use of underclothing and socks made of silk, a fabric repugnant to lice; frequent bathing and change of clothing ("after clothes have been discarded for a week the lice are usually dead of starvation"—Shipley); boiling or burning clothing known to be infected.

[1] Maxcy: Public Health Reports, 1926, 41[2], p. 2967.

[2] "Those near him know that he fully understood the dangers to which he would be exposed and the risks he would run. He decided he would take those risks, meet the dangers with all possible means of prevention, and do the work that would come to his hands. And so he made the great sacrifice and gave all that a man can give to his fellow-men."—Hektoen.

Lice may be killed with kerosene, nits (eggs) with vinegar. A variety of body ointments have been used, with more or less success. A lather of dilute cresol soap applied to the skin and also allowed to dry on the inside of garments has been effective for protecting soldiers from lice under war-time conditions (Lancet, February 6, 1915). Powdered sulfur sprinkled over the skin is said to repel the vermin (from the generation of H_2S?), but the efficacy of this procedure has been questioned. In the great Serbian epidemic of 1915 it is said that "measures looking to the eradication of lice from people and from their habitations, the improvement of personal and community cleanliness and hygiene, and the isolation of people afflicted with the disease, within a short time placed the epidemic under complete control."[1]

The serum of typhus convalescents possesses specific preventive properties, and Nicolle[2] has prepared an antityphus serum from asses which is said to have given favorable results in human typhus. The ass is injected intravenously with emulsions of leukocytes from infected guinea-pigs, followed by similar injections with emulsions of infected guinea-pig spleen. The serum has preventive as well as curative properties.

The Weil-Felix Reaction.—In 1915 two strains of Proteus isolated from typhus cases were found by Weil and Felix[3] to be markedly agglutinated by the serum from these and other typhus patients. A little later (1916) the same workers found another strain (Proteus X19) that agglutinated in still higher dilutions. This strain has been extensively employed in testing serum from typhus and suspected typhus patients, using an agglutination technic similar to that used for the Widal reaction. In practically all cases tested the Weil-Felix reaction has proved of diagnostic value. Ordinary strains of saprophytic Proteus and other bacilli such as the typhoid bacillus are not agglutinated by typhus serum, and conversely Proteus X19 is not agglutinated by the serum from typhoid fever, meningitis, and other diseases.[4] The significance of the agglutination of Proteus X19 by typhus serum is not clear. There is little reason to suppose that the organism has any causal relation to the disease. Perhaps the simplest explanation is that

[1] Caldwell: Jour. Amer. Med. Assoc., 1916, 66, p. 326.
[2] Nicolle and Blaizot: Ann. de l'Inst. Past., 1916, 30, p. 446.
[3] Weil and Felix: Wien. klin. Wchnschr., 1916, 29, pp. 33, 297.
[4] Ida A. Bengtson: Public Health Reports, 1919, 32^2, p. 2446.

the Proteus is a secondary invader, but its relatively infrequent isolation in some hundreds of cases and its apparent lack of marked pathogenic properties count against this view. Some writers are of the opinion that the sera of typhus cases are especially rich in secondary agglutinins.

The Specific Organism.—The virus of typhus is present in the circulating blood during the febrile period, as was shown by Moczut-

Fig. 171.—A venule of the skin showing a compact cluster of small paired rickettsiæ. The vessel shows an early lesion, the endothelium is swollen and has taken up red corpuscles. The intima is invaded by leukocytes. There is also a perivascular accumulation of endothelial leukocytes (Wolbach, Todd. and Palfrey).

kowski, who developed a typical case of the disease after inoculating himself with typhus blood.[1] Nicolle,[2] Ricketts and Wilder,[3] and Anderson and Goldberger[4] obtained similar results in animal experiments and were able to transmit the disease to various species of monkeys. Gaviño and Girard[5] report success in inoculation of

[1] Moczutkowski: Allg. med. Centr.-Ztg., 1900, 69, p. 1055.

[2] Nicolle: Compt. rend. Acad. Sci., July 12, Sept. 6, 1909.

[3] Ricketts and Wilder: Jour. Amer. Med. Assoc., 1910, 54, pp. 1304 and 1373; 1910, 55, p. 309.

[4] Anderson and Goldberger: Public Health Reports, Dec. 10 and 24, 1909; Feb. 4 and 18, 1910; Jour. Med. Research, 1910, 22, p. 469.

[5] Gaviño and Girard: Pub. Instit. Bact. National Univ., Mexico, 1911.

the guinea-pig with typhus virus even to the extent of eleven passages through the animals.

The earlier statements on the filterability of the typhus virus were conflicting, but it seems now to be established that the virus does not pass through a Berkefeld filter. Inoculation with filtered blood does, however, render monkeys refractory to further infection, according to the testimony of several observers.

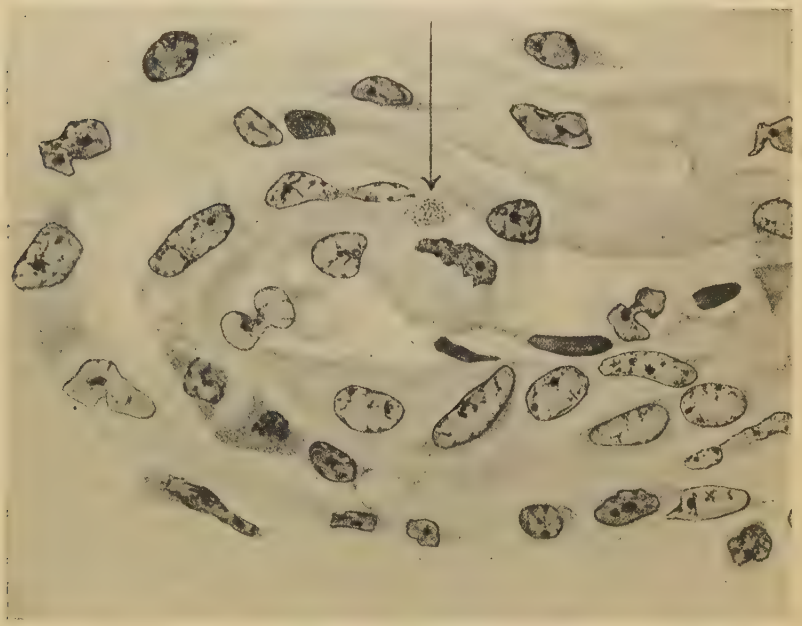


Fig. 172.—Louse Box 54, Louse 1. *Rickettsiæ prowazeki.* 1800 diameters. Section. Swollen epithelial cell of the mid-gut containing bacillary forms of Rickettsiæ. The organisms are usually arranged in skeins of thread-like chains. They also occur singly, in pairs, and in short chains. Lying free in the lumen of the gut is a short Rickettsia thread (Wolbach, Todd, and Palfrey).

The minute bacterium-like organisms found in human tissues (Fig. 171) and in lice fed on typhus patients (Fig. 172) are generally considered distinct from the ordinary bacteria and classed in the genus Rickettsia. These micro-organisms were first observed by Ricketts in lice. The species found in typhus is R. prowazeki, the specific name honoring the memory of the distinguished protozoölogist Prowazek, who, like Ricketts, lost his life in studying typhus.

The rickettsia bodies stain with difficulty by the ordinary staining methods used for bacteria; Giemsa's solution gives the most satisfactory results. R. prowazeki has never been cultivated

and hence little is known about it outside its morphological characters. Wolbach, Todd, and Palfrey[1] marshall an imposing array of evidence in support of its causal relation to typhus fever. Lice fed on typhus patients sometimes but not always acquire R. prowazeki. In all cases, however, it is found that lice shown to contain the virus of typhus also harbor R. prowazeki. Bodies indistinguishable from R. prowazeki are demonstrable with great regularity in the lesions of typhus in man. Especially significant is the fact that lice taken from a region free from typhus and proved by examination not to contain rickettsiæ acquire the organisms when fed on typhus patients and then become capable of transmitting the disease.

Rickettsiæ in Other Connections

Trench Fever.—In the course of the Great War a specific infection became known under the name of trench fever or Wolhynian fever. It is said to have caused almost one-third of all the sickness in some of the armies in northern France and it occurred also in the armies in Mesopotamia and Saloniki. It has a long incubation period (six to twenty-two days); the most constant symptom is said to be pain in the legs; the fever is often high and of the relapsing type. Recovery is the rule. The disease can be transmitted to healthy men by the intravenous injection of whole blood taken from patients up to the fifty-first day of the disease.

Natural transmission is chiefly, if not solely, through the body louse. Bites alone from infective lice appear not to produce trench fever, but the virus is present in the excreta of these vermin and enters through abrasions in the skin such as are caused by scratching. The virus is present in the urine of trench fever patients and is said to remain active for a long time in dry louse feces and dried urine.

The studies of Arkwright, Bacot and Duncan[2] established the fact that in most cases lice or the excreta of lice proved by inoculation to contain the virus contained large numbers of rickettsiæ. There is other evidence. Bacot who later became accidentally infected with trench fever had previously been feeding upon his person a stock of lice known to be free from rickettsiæ for over two years; on

[1] Wolbach, Todd, and Palfrey: "The Etiology and Pathology of Typhus," Cambridge, 1922, p. 222.

[2] Arkwright, Bacot and Duncan: Jour. Hyg., 1919–20, 18, p. 76.

resuming the feeding of these lice two days after the onset of his attack, rickettsiæ were observed eight days afterward in enormous numbers in the excreta of these vermin. Infection of the lice with rickettsiæ continued possible for as long as three months after the disappearance of all symptoms. Nothing, however, is certainly known about the occurrence of rickettsiæ in the bodies of patients with trench fever. Further evidence is necessary to establish the causal relation of Rickettsia pediculi to trench fever although there is a strong presumption that such a connection obtains.

Heartwater Disease.—In a highly fatal disease of cattle, goats and sheep in South Africa, Cowdrey has found an organism (R. rumantium) which appears to be the cause of the infection.[1] It is transmitted by ticks, and experimentally produced heartwater disease shows that the presence of rickettsiæ coincides in all cases with the presence of the heartwater virus.

Besides the several presumably pathogenic forms of rickettsiæ just described, nearly forty presumably non-pathogenic micro-organisms of this group have been found in various species of insects and arachnids. Little is known about them. Their presence in some seemingly normal insects with no known connection with disease adds to the difficulty of detecting and studying pathogenic types. Noguchi has succeeded in cultivating one of the non-pathogenic rickettsia-like organisms from the Rocky Mountain spotted fever tick.[2]

[1] Cowdrey: Jour. Exper. Med., 1925, 42, pp. 231, 253.

[2] Noguchi: Jour. Exper. Med., 1926, 43, p. 515.

CHAPTER XXXIV

THE BACTERIOLOGY OF MILK AND MILK PRODUCTS

It is a familiar observation that milk sours on standing. The agency of bacteria in this, one of the earliest known fermentive processes, was established by the work of Pasteur in 1857. It was first shown by Hueppe in 1884 that a particular species of micro-organism was usually associated with the process.

The Fermentation of Milk.—Lactic fermentation consists in the conversion of milk sugar or lactose into lactic acid (Ger., *Milchsäure*). Lactose itself is not directly fermentable, but must first be converted into the simpler sugars, glucose and galactose. The equation,

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} = \underset{\text{Lactic Acid}}{4C_3H_6O_3}$$

although substantially correct, has only an approximate value.

The quantity of acid necessary to effect the curdling of milk (precipitation of the casein) varies somewhat according to the amount of casein and phosphate present, but averages about 0.45 per cent. The terminal acidity may go much higher than this (0.85 per cent.). Sometimes the coagulation of milk takes place in the presence of a relatively small amount of acid, especially if the milk is boiled or pasteurized. In general, the curdling of milk depends upon degree of acidity, temperature, time of action, amount and solubility of calcium salts present, and other factors. The heat generated in the spontaneous souring of milk is far greater than could come from the lactose fermentation alone; the process is, therefore, a complicated one.

Many different species of bacteria are able to provoke the lactic fermentation, among them such familiar organisms as Staphylococcus aureus, Streptococcus pyogenes, and B. coli. A few species are commonly responsible, however, for the natural souring of milk. The common lactic-acid bacteria may be divided into two groups. One of these comprises capsulated gas-forming bacilli of the B. (lactis) aërogenes type. These organisms are closely related to B.

coli, differing principally in their possession of capsules, in their lack of motility, and their ability to produce gas from potato starch. (See p. 313.)

The second type is a streptococcus to which the name Streptococcus lacticus has been given (Kruse[1]) (Fig. 173). This streptococcus is very abundant in naturally soured milk, particularly when the acidity has reached a high point. Streptococcus lacticus has been found by Heinemann on the skin of cows, in cow-dung, and in milk at all stages of handling. The milk streptococcus in all its

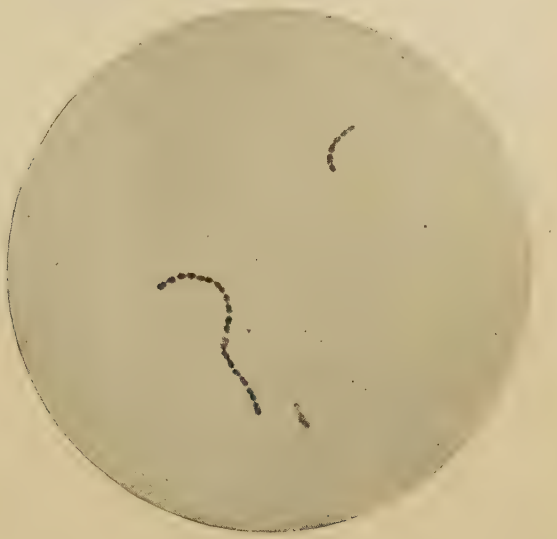


Fig. 173.—Streptococcus lacticus from serum broth (Heinemann).

properties is extraordinarily like Streptococcus pyogenes (Heinemann[2]). As the result of a comprehensive investigation Heinemann concludes that both B. aërogenes and Streptococcus lacticus are ordinarily present in naturally souring milk, the former species in abundance in the beginning of the fermentation, the latter in the later stages. Inoculation of sterilized milk with pure cultures of either one of these two organisms and with mixtures reproduces very closely the process of natural souring.

[1] Leichmann ("Milchzeit." 1899, 23, p. 523) was the first to demonstrate the importance of this group of organisms, although he erroneously regarded them as bacilli, and gave them a name (Bacillus lactici acidi) which has been the source of much confusion.

[2] Heinemann: Jour. Infect. Dis., 1906, 3, p. 173.

Although milk allowed to stand ordinarily turns sour from the formation of lactic acid, other fermentations are exceptionally observed. The butyric acid fermentation

$$\underset{}{C_6H_{12}O_6} = \underset{\text{Hydrogen}}{2H_2} + \underset{\text{Carbon Dioxide}}{2CO_2} + \underset{\text{Butyric Acid}}{C_4H_8O_2}[1]$$

is one of the most thoroughly studied, and can be caused by a number of different, but closely related, anaërobic bacteria. Several well-known pathogenic anaërobes, such as the bacilli of symptomatic anthrax and of malignant edema (both non-motile), are able to produce butyric acid. Schattenfroh and Grassberger,[2] who have made a particular study of this fermentation, describe also a "motile butyric acid bacillus" which, like the two non-motile forms above mentioned (non-motile butyric acid bacilli), is widely distributed in nature, but, unlike them, is not pathogenic. A distinction is also made between those bacteria that are able to produce butyric acid from carbohydrates (and to some extent from lactic acid and glycerol) and those that produce butyric acid accompanied by the generation of malodorous gases in the course of protein decomposition. To the former class belong the anaërobes already mentioned; to the latter, certain aërobes closely allied to the hay bacillus or potato bacillus.

The spontaneous alcoholic fermentation of milk is less usual under natural conditions than either the lactic or butyric. The manufacture of certain alcoholic beverages is, however, dependent upon the artificial production of this form of milk fermentation. A well-known drink called koumiss is made by Tartars from mare's milk, a small quantity of old koumiss often being added to fresh milk as a starter. The bacteriology of the process is not known. The similar beverage known as kefir, an effervescent sour milk prepared by the inhabitants of the Caucasus from the milk of cows, goats, and sheep, is made with the aid of "kefir grains," small, irregular, yellowish granules of a gelatinous consistency. The microbic composition of the kefir grain has been studied by a number of investigators, with rather discordant results.[3] Several species of bacteria have been described as present, but it does not seem

[1] The actual process is much more complex than indicated by this equation.

[2] Schattenfroh and Grassberger: Arch. f. Hyg., 1900, 37, p. 54.

[3] Beijerinck: Centralbl. f. Bakt., 1889, 6, p. 44; v. Freudenreich: Landw. Jahrb. d. Schweiz, 1896, 10, p. 1.

probable that all the organisms found in kefir grains are necessarily concerned in the specific fermentation process. According to Nikolaiewa,[1] only two micro-organisms (B. caucasicus and Torula kefir) are essential, and all the others are to be regarded as accidental contaminations. B. caucasicus is a long, slender, non-motile bacillus which produces great quantities of lactic acid from milk-sugar. The yeast organism ferments lactose, glucose, and sucrose, and is apparently responsible for the alcoholic part of the fermentation. By the symbiotic use of these two species of micro-organisms in pure cultures Nikolaiewa succeeded in preparing the characteristic kefir beverage. In such processes, however, the action of bacteria is not always indispensable. Some species of yeasts are able to effect the alcoholic fermentation of milk in pure culture.

A variety of other fermentations of milk may be sometimes caused by bacteria. Casein may be precipitated by rennet of bacterial origin (Conn[2]) or dissolved by bacterial casease. The behavior of pure cultures of various micro-organisms inoculated into sterilized milk gives evidence of widespread ability to provoke fermentation changes. Many cultures cause acid production and consequent precipitation of the casein, which is then slowly dissolved (casease); others dissolve the casein without initial precipitation, and still others curdle the milk without acidity.

A series of unusual or abnormal changes, sometimes called "diseases" of milk, are produced by certain bacteria which occasionally find their way into milk. "Blue milk" (B. cyanogenes), "red milk" (B. prodigiosus, B. erythrogenes, *et al.*), and "yellow milk" (B. synxanthus) are caused by the presence of various chromogenic organisms. The bitterness that sometimes develops in milk after a short interval is due to the products of certain micro-organisms. Several different bacteria have been met with in outbreaks of this not very uncommon dairy trouble. Harrison[3] found that a yeast-like organism (Torula amara) was apparently the active agent in an epidemic of bitter milk in Canada. There seems little doubt that a number of species are capable of imparting a bitter taste to milk and its products, although nothing is known of the chemical nature of the substances concerned. Milk sometimes suffers also

[1] Nikolaiewa: Centralbl. f. Bakt., II, 1908, 21, p. 161.
[2] Conn: Storrs Agri. Exp. Sta. Rep., 1892, 5, p. 106.
[3] Harrison: Centralbl. f. Bakt., II, 1902, 9, p. 206.

from a ropy or slimy fermentation which, under most circumstances, is considered undesirable and has caused great loss to the butter industry in Switzerland and elsewhere. Thorough disinfection of the utensils and premises is usually sufficient to stamp out all these anomalous and in some instances disastrous fermentations. For certain purposes, as in the manufacture of Edam cheese in Holland, slimy milk is intentionally produced by use of a particular species of streptococcus.

Methods of Bacterial Examination of Milk.—The need for controlling the character of the public milk supply has led to the development of special methods of laboratory examination. These have been tested and simplified for routine purposes by the cooperation of many laboratory workers in this country and are set forth in detail in a special laboratory guide prepared under the auspices of the American Public Health Association and other interested organizations.[1]

Samples of milk (at least 10 c.c.) are taken with a sterile tube after thorough mixing in the original container. For transportation they must be transferred to a tightly stoppered vial or bottle and iced. If possible plates should be poured within four hours. The standard agar medium with a P_H between 6.2 and 7.0, preferably 6.6, should be used. Three dilutions—1 : 100, 1 : 1000 and 1 : 10,000—are ordinarily plated. The standard incubation period is 48 hours at 37° C. As in water analysis the colony count has only a relative significance, since with the most favorable medium all the viable bacteria in the milk do not develop. The colony count or "standard plate count" is assigned greater importance in the examination of milk than in that of water, no enumeration of specific bacteria—as of B. coli in water examinations—having yet been shown to possess much value.

Besides the ordinary macroscopic colony count obtained by plating, a microscopic method devised by Breed[2] is of value for many purposes. In the Breed method milk is taken in a capillary pipet discharging 0.01 c.c., and is dried over an area of one square centimeter on a microscopic slide. After washing out the fat with xylol and fixing with alcohol, the film is stained with Löffler methylene blue. The number of bacteria per c.c. is estimated by counting

[1] Standard Methods of Milk Analysis, 5th Edition, 1927.
[2] Breed: N. Y. Agri. Exp. Station Bull., No. 443, 1917.

a carefully measured area. The ratio used in comparing the microscopic count with the standard plate count is 4 : 1. The direct microscopic count has proved of special value in judging the quality of fresh milk as delivered at pasteurizing plants or other milk receiving stations.

Sources of Bacteria in Milk.—Freshly drawn milk is not sterile, but always contains some bacteria. Under ordinary conditions these come in part from the surface of the udder, from the hands of the milker, and other outside sources, but always in part also from the udder itself. After much conflicting testimony, it is now well established that the milk in the udder of the cow is rarely, if ever, germ-free, but that the germ-content is different in different animals, and that the first portion of the milk drawn (fore-milk) always contains more bacteria than the last (strippings). The milk secreted by healthy milk-glands is, as a rule, sterile, but the milk-ducts in the teats of the cow afford a ready pathway for the invasion of the udder. The bacteria that abound in the milk-ducts would probably grow back into the udder more freely than they do if it were not for the germicidal power possessed by milk, as by other body-fluids. The existence of a germicidal property has been called in question,[1] and the death of bacteria falling into milk ascribed simply to their inability to grow in that medium; but even those kinds best adapted for growth die off at first when introduced into perfectly fresh milk. Most investigators agree that milk, like serum, possesses a genuine although feeble germicidal power.[2] The action is different for different species of bacteria, and the milk from one animal may be more actively bactericidal than that from another. At best, the germicidal property can never be relied upon to take the place of cleanliness and icing.

When milk is collected under ordinary conditions, the udder germs form but an insignificant fraction of the total number of bacteria in the milk. The skin of the cow, the hands of the milker, the vessels used for collection, and the dust of the cowbarn all contribute their quota to the number found immediately after milking. If milk is obtained with aseptic precautions, it contains only a few hundred (200 to 400) germs in a cubic centimeter. Collected with

[1] Stocking: Storrs Agri. Exp. Sta. Rep., 1904.

[2] Rosenau and McCoy: Jour. Med. Res., 1908, 18, p. 165; Jones and Little: Jour. Exper. Med., 1927, 45, p. 319.

somewhat less care, it may contain a few thousand (2000 to 6000); with careless manipulation milk, even when freshly drawn, may be highly contaminated (30,000 to 100,000 per c.c.). Milk is a particularly favorable medium for bacterial growth, and when it is richly seeded at the outset, enormous multiplication will occur, provided it is kept at ordinary summer temperatures. The temperature factor is indeed all important. If milk is kept at 0° C. (32° F.), it shows a decrease in the bacterial content during the first 168 hours, but at higher temperatures the rate of multiplication is prodigious.

Milk Collected with Great Care[1]

Initial content, 3000	Kept at:			
	4° C.	6° C.	13° C.	20° C.
24 hours	2,500	3,100	18,800	450,000
48 "	3,600	12,000	3,400,000	....
96 "	218,000	1,480,000	....	....
168 "	4,209,000	80,000,000	....	....

Ordinary Milk

Initial content, 30,000	Kept at:			
	4° C.	6° C.	13° C.	20° C.
24 hours	38,000	42,000	187,000	4,000,000
48 "	56,000	360,000	38,000,000	....
96 "	4,300,000	12,200,000	....	....
168 "	38,000,000	300,000,000	....	....

Many such figures might be cited, but they all lead to the same conclusion: namely, that the number of bacteria in milk depends chiefly—(1) upon the degree of original contamination of the milk, (2) upon the age of the milk, and (3) upon the temperature at which it has been kept. In other words, the bacterial count gives valuable information both as to the cleanliness and staleness of this indispensable food. All observations emphasize the importance of rapidly chilling the milk as soon as possible after it is drawn, and of keeping it constantly at as low a temperature as possible. Milk collected in sterilized vessels with proper precautions, and then

[1] Adapted from Park, "Pathogenic Bacteria," N. Y., 1905, p. 463.

cooled and kept cool, has been preserved in a thoroughly sweet and fresh state for periods that seem surprisingly long judged by ordinary household experience. Bottles of such clean and cool milk, without addition of any preservatives, have been transported from the vicinity of Chicago to Europe without losing either palatability or wholesomeness.

Top milk and gravity cream contain more bacteria than skim milk since bacteria rise with the cream. Fresh separator cream contains, as a rule, fewer bacteria than the original milk. In the process of centrifugalization, the bacteria, being heavier than water, tend to drop to the bottom, while the cream, being lighter, rises to the top.

It is a matter of common knowledge that before the introduction of pasteurization much of the milk distributed in large cities was too far advanced in bacterial decomposition to be a desirable food. In New York City it was found by Park[1] that during the coldest weather the milk in the shops averages over 300,000 bacteria per cubic centimeter; during cool weather, about 1,000,000, and during hot weather, about 5,000,000. In Chicago Heinemann and the writer[2] found in market milk collected during April, May, and June numbers ranging from 10,000 to 74,000,000; and in Boston Sedgwick and Batchelder[3] reported that samples of milk from groceries averaged over 4,500,000.

The question has often been raised whether there are not certain kinds of bacteria that invariably predominate in milk; whether, in short, it is not desirable to recognize the existence of a special class of milk bacteria. Swithinbank and Newman[4] give descriptions of some one hundred and twenty "milk bacteria," the statement being expressly made that "organisms of water, soil, etc., are not included." Other writers also appear to believe that certain species are especially adapted for life in milk, and habitually gain the upper hand in this fluid under natural conditions. As a matter of fact, there is little to recommend this view. Fresh milk is an admir-

[1] Park: Jour. Hyg., 1901, 1, p. 391.

[2] Report of the Civic Federation of Chicago, 1904.

[3] Sedgwick and Batchelder: Boston Med. and Surg. Jour., 1892, 126, p. 25. This paper is the first report made upon the bacterial content of milk in an American city.

[4] Swithinbank and Newman: "Bacteriology of Milk," New York, 1903, pp. 392–451.

able medium for the growth of a great many species of bacteria, as reference to lists of "milk bacteria" will show, and there is no reason to consider it as preëminently favorable for a few special kinds. If some varieties are found in milk more commonly than others, this is in large part, if not altogether, because they are more commonly present in the environment in which the milk is collected. The dependence of the flora of milk upon the bacterial environment has been noted by many observers. The dust of the cow-stable, the nature of the straw used for bedding, even the character of the pasture, have been observed to affect the kind and abundance of the species found in milk. Weigmann and Zien[1] have shown that the use for bedding of a poor quality of straw, which is almost always full of molds—so-called wild yeasts—and peptonizing bacteria, is likely to be followed by undesirable fermentations in milk. On the whole, it would seem that the kinds of bacteria found in milk are more likely to be determined by the opportunities of contamination to which the milk is exposed, and the temperature at which it is kept, than by any special adaptability to this particular food medium.

The entrance of pathogenic bacteria into raw milk sometimes occurs. Both individual cases and epidemics of several infectious diseases have been clearly traced to the drinking of uncooked milk Two ordinary sources of infection are recognized: the germs may be derived directly from the cow, or they may be introduced into milk with infectious material of human origin. The latter is much the more common source. The bacteria of typhoid fever, of cholera, of diphtheria, of scarlet fever and of septic sore throat may find their way into milk through the agency of convalescents or persons suffering from mild attacks of these diseases who are engaged in the milking process, or in the handling of milk and milk products.[2] Sometimes, too, infections may come about in a more circuitous fashion, as exemplified in the causation of typhoid fever by the use of contaminated water for rinsing milk-cans or other utensils. A serious source of danger lies in the improper disposition of excreta in country privies and in the fact that flies may convey typhoid bacilli from the excreta to milk (pp. 338, 339, 340).

[1] Weigmann and Zien: Milchzeit, 1893, 22, p. 569.

[2] See "Milk and Its Relation to the Public Health," Bull. 41, Hyg. Lab., Pub. Health and Mar. Hosp. Service, 1908, pp. 19–159.

Tuberculosis attributed to milk is commonly assumed to be due to bacilli of bovine origin, but it is also possible for milk to become contaminated with tubercle bacilli from tuberculous persons. It is a not unknown practice in country districts for milkmen to begin the milking process by moistening their hands with saliva. Milk may also conceivably become contaminated with human tubercle bacilli by means of the "infectious droplets" discharged in coughing or sneezing. Owing to the difficulty of tracing a case of tuberculosis to its origin, the degree of danger from this source is uncertain, although the possibility of contamination through tuberculous milkers should not be overlooked. The relation of bovine tuberculosis to human tuberculosis is elsewhere considered (pp. 444–446).

Among other diseases of the cow transmissible to man through the medium of milk, may be mentioned foot-and-mouth disease (p. 612). Extensive outbreaks of this affection, which attacks especially children, have been caused by the use of raw milk. In fact, milk, butter, and cheese are the usual, if not the exclusive, vehicles of this malady.

Inflammation of the udder of cows (garget or mastitis), a condition that may be provoked by a variety of organisms, is apparently fraught with some danger to the consumer of raw milk. On more than one occasion the use of milk from udder-sick cows has been followed by illness, but the bacteriology of these cases has not been always determined. The same must be said of the association of enteritis in cows with gastro-intestinal troubles in man. The epidemiologic connection between sudden attacks of illness and the use of the milk of cows suffering from diarrhea is undoubted, but the nature of the micro-organisms (or toxins?) concerned has remained in many instances uncertain. Streptococci have been regarded as the cause of some of these outbreaks, paratyphoid bacilli and organisms resembling B. coli of others.

Some other affections to which cattle are liable seem of less importance. Actinomycosis attacks the cow's udder not infrequently, but not a single case of this disease in man has ever been shown to have been communicated by milk. Anthrax likewise is not ordinarily and directly a milk-borne disease, probably in part, at least, because anthrax bacilli are not able to pass from the circulation into the milk of an infected animal except in the later and

easily recognizable stages of the disease. It is not known that rabies or the pleuro-pneumonia of cattle has ever been conveyed to man by milk, although if injuries of the mouth or digestive tract exist, rabic infection by this channel is theoretically conceivable. Goat's milk has been shown to be the chief medium of infection in undulant or Malta fever. (See p. 354.) Contagious abortion of cattle is caused by Brucella abortus, an organism closely similar to and perhaps a variety of the germ of undulant fever (Brucella melitensis). Human infection with Br. abortus was definitely reported in 1924.[1] A number of other cases of undulant fever due to this organism have since come under observation, many of them in persons who had drunk heavily of raw milk.[2] At the present writing human infection with Brucella abortus seems to be rare especially in temperate countries, but final judgment as to its frequency must be suspended. In view of all these manifold, if not yet clearly understood, possibilities of infection, it would seem an indispensable precaution to avoid the use of uncooked milk from an animal with any symptom of illness whatever.

Infant Diarrhea.—The occurrence of "infant" or "summer" diarrhea in young children whose food consists wholly or in large part of cow's milk has been the subject of much investigation. The predominant influence that the kind of feeding has upon this condition is shown by numerous comparisons that have been made between the sickness and mortality among breast-fed children and among those fed with condensed milk or cow's milk. The following figures (from Park) are typical of the difference observed everywhere:

	Did Well.	Did Fairly.	Did Badly.	Died.	Total.
Store milk	21	23	20	15	79
Condensed milk	22	20	14	14	70
Best bottled milk	9	3	0	0	12
Breast feeding	17	7	7	0	31

In Berlin, during the five years, 1900–1904, there were 41,383 deaths of infants whose method of feeding was ascertained; only 3995 of these were breast-fed; in other words, more than nine-tenths of the infant mortality occurred among those fed artificially.

[1] Keefer: Bull. Johns Hopkins Hosp., 1924, 35, p. 6.

[2] Alice Evans: Jour. Amer. Med. Assoc., 1927, 88, p. 630.

Harrington[1] has pointed out that infant mortality is a class mortality, highest, as a rule, in those cities and towns where women work in industrial establishments and put their children early to the bottle.

The bacteriology of infant diarrhea is itself by no means definitely established. Some observers would lay the responsibility at the door of certain streptococci very similar to, if not identical with, Streptococcus pyogenes, but this view has not met with general acceptance. More recently, varieties of the dysentery bacillus have been connected with summer diarrhea, and have been found in large numbers and repeatedly in the stools of typical cases by a number of observers.[2] It is not necessary to assume, however, that identical clinical symptoms are invariably caused by one and the same micro-organism. Booker,[3] one of the most indefatigable workers in this field, expressed the opinion that "no single micro-organism is found to be the specific exciter of the summer diarrhea of infants, but the affection is generally to be attributed to the result of the activity of a number of varieties of bacteria." Park and Holt, as the result of a comprehensive study of the relation between milk-supply and infant diarrhea in New York City, reached the conclusion that "no special varieties of bacteria were found in unheated milk which seemed to have any special importance in relation to the summer diarrhea of children."[4] While it is therefore perhaps premature to assign a uniform bacterial cause for every case of infant diarrhea or to attempt to differentiate between different causes, there can be no doubt as to the influence of the numbers of bacteria in milk. The most conclusive investigations on this point are those of Park and Holt. These observers found that during hot weather the effect of bacterial contamination on the health of infants was very marked when milk was fed without previous heating. "When milk is taken raw, the fewer the bacteria present the better the results. Of the usual varieties, over 1,000,000 bacteria per cubic centimeter are certainly deleterious to the average infant."

It should be remembered that when once the specific agent of intestinal trouble, whatever it be, is introduced into the gastro-

[1] Harrington: Amer. Jour. Med. Sci., 1906, 132, p. 811.

[2] Studies from the Rockefeller Inst. for Medical Research, 2, 1904.

[3] Booker: Johns Hopkins Hosp. Rep., 1896, 6, p. 159.

[4] Park and Holt: Archives of Pediatrics, Dec., 1903, 20, p. 881.

intestinal canal of infants, the continued use of milk from any source is injurious. If the resistance of an infant is depressed by hot weather, the effect of abnormal fermentation and putrefaction of the milk within the body may be especially serious. The factors involved in the causation of summer diarrhea in infants are varied and complex.

Certified Milk.—One of the earliest attempts to avoid the dangers of milk-borne infection was the elaboration of methods designed to safeguard milk at every step in its production, collection and distribution. To this end "Medical Milk Commissions" were established in a number of localities in the United States, usually under the auspices of the local medical society. Milk conforming to certain standards is certified by such a Commission to be of high quality. The regulations, which are generally excellent, deal with such matters as the cleanliness of barnyard and dairy; the purity of the farm water supply; the proper sterilization of utensils; and the health of the cows and of the milkers. Especial attention is paid to the bacterial content of the milk, for which a maximum limit of 10,000 germs per cubic centimeter is set. Certified milk is undoubtedly safer to use than milk collected and transported without suitable supervision, and the work of the milk commissions has done much to improve dairy conditions in many parts of the country. At the same time raw milk, certified or not, can never be looked upon as beyond all chances of contamination. The difficulty—not to say impossibility—of making sure that no typhoid carriers, and no person suffering from a mild case of diphtheria or scarlet fever are ever employed in a dairy is often urged as an objection. In point of fact outbreaks of diphtheria, paratyphoid fever and other diseases have been traced to certified milk. For this reason as well as because of the relatively high cost of production the use of certified milk remains limited.

Pasteurization.—The use of a temperature high enough to kill most micro-organisms but not so high as to produce radical alterations in organic substances was first applied by Pasteur to preserving wines without destroying their original flavor or bouquet. At the present day the method of pasteurization is chiefly used for the treatment of milk. Numerous epidemics of disease were formerly traced to milk. Up to 1927, there were actual records of 613 outbreaks of milk-borne typhoid in the United States alone—65 of

scarlet fever and 43 of diphtheria besides many outbreaks of septic sore throat and other infections.[1] The amount of unidentified disease in infants and young children caused by raw milk cannot be estimated.

The general pasteurization of the milk supply of the larger American cities has greatly diminished milk-borne disease. In 1924 over 90 per cent. of the milk supply in all the largest cities (over 500,000 population) was pasteurized and more than 50 per cent. in most of the smaller cities (over 25,000 population). The proportion of pasteurized milk used throughout the country seems to be steadily increasing. Corresponding with the growth of pasteurization the amount of disease traced to milk has dwindled to a low point. In 1907–1915 in Massachusetts 2215 typhoid cases were traced to milk; in 1919–1923, only 297.

Some of the earlier types of pasteurizing machines were quite defective and failed to achieve their purpose. This was particularly true of the "flash" process where the machine was designed to expose milk for a brief period to a relatively high temperature. The use of a lower temperature continuously for a longer period as exemplified in the "holding" process is now general. Even so, the existence of defects in certain types of modern pasteurization must be squarely faced.[2]

The aim of pasteurization being primarily the destruction of pathogenic organisms in the milk, there has been much experimentation and discussion as to the most suitable temperature and time of exposure. Current requirements usually specify exposure for 30 minutes either to 142° or 145° F. (62.2–63.9 C.). Experiments indicate that the tubercle bacillus, probably the most resistant of the pathogenic bacteria likely to be found in milk, is killed by exposure to 140° F. (61.1 C.) for 20 minutes,[3] and at 136° for 30 minutes[4] so that a temperature of 142° F. (for 30 minutes) should allow an ample margin of safety. In practice the difficulty has arisen of insuring that "every particle of milk" be held at the given temperature for the required period. Objection to the use of a temperature of 145° F. has been made on the ground that if "every

[1] Armstrong and Parran: Public Health Reports, Suppl. No. 62, 1927.

[2] Commercial Pasteurization. Pub. Health Bull., No. 147, Washington, 1925; Putnam: Am. Jour. Pub. Health, 1927, 17, p. 121.

[3] Park: Am. Jour. Pub. Health, 1927, 17, p. 36.

[4] North and Park: Am. Jour. Hyg., 1927, 7, p. 147.

particle" of milk be heated to at least this temperature some particles will inevitably be heated to temperatures as high as 147° or 148° F. and that these high temperatures will destroy the creaming ability of the milk. Many sanitary authorities believe that the destruction, or even the material reduction of the creaming ability of commercially pasteurized milk, would cause great prejudice against the pasteurizing process. On the other hand, there are those that believe that any temperature lower than 145° F. (63.9 C.) does not allow a sufficient margin of safety. Whatever the outcome of the controversy, it is clear that one of the immediate needs is the correction of mechanical defects in the existing types of pasteurizing devices so that "cool pockets," dead ends and leaky valves are eliminated. When this has been done it may be found that a temperature of 142° F. affords an entirely adequate safety margin.[1]

The general decline in milk-borne disease, including bovine tuberculosis, which has occurred in communities where pasteurization has been put into effect, indicates that whatever may be the shortcomings of some forms of pasteurizing equipment, the general introduction of the process has greatly lowered the danger of infection. Relatively little disease is now traced to milk in our large American cities. Supervision of commercial pasteurization is, however, essential, not merely to detect defects in machinery but to insure the proper conduct of the whole process. In the extensive Montreal 1927 typhoid outbreak it was thought that only part of the milk entering a large pasteurizing plant was subjected to pasteurization, the balance being distributed to the consumers in a raw condition.[2]

Not all bacteria, of course, are killed by pasteurization.[3] Besides the resistant spore-formers, aërobic and anaërobic, certain streptococci are able to survive the pasteurizing temperature. All the known disease-producing streptococci, however, appear to have a low thermal death point. Milk pasteurized at the usual temperatures (62°–64° C. or 142°–145° F.) sours in the ordinary way on standing, but if higher temperatures be used (*e. g.* 180° F. or 82.2° C.) the lactic acid bacteria are killed. In such cases the

[1] Frank, Moss and LeFevre: Pub. Health Reports, 1927, 42, p. 1152. Putnam: Am. Jour. Pub. Health, 1927, 17, p. 121.

[2] Jour. Amer. Med. Assoc., 1927, 89, p. 217.

[3] *Cf.*, *e. g.* Prucha: Am. Jour. Pub. Health, 1927, 17, p. 356.

peptonizing bacteria which survive this temperature cause the milk to decompose.

The high food value of milk makes it desirable to keep the expense of producing safe milk as low as possible. It has been estimated that the average cost of pasteurizing one gallon of milk is a little less than one-half cent per gallon.[1]

Current objection to pasteurization is mainly on the ground of vitamin destruction. Vitamins A (fat-soluble) and B (water-soluble), both abundant in milk, are quite resistant to heat, but the antiscorbutic vitamin C is weakened or destroyed by pasteurizing temperatures. Infants fed exclusively on a diet of pasteurized milk will develop scurvy. Fortunately the addition to the diet of readily accessible antiscorbutics such as orange juice or tomato juice is tolerated by infants much earlier than used to be supposed, even in the first month of life. The reasonable procedure, therefore, appears to be to use pasteurized milk to insure protection against disease germs of various kinds and to meet the vitamin deficiency with other agents. The success in infant feeding based on this principle is evinced especially in the amazing reduction in infant mortality in the summer months.

It is self-evident that pasteurization should not be used to conceal slovenly methods of milk production. To this end limits as to bacterial content before and after pasteurization are established by many city health departments. In New York City "Grade A" milk must not contain more than 200,000 bacteria per c.c. before, or more than 30,000 after, pasteurization; "Grade B" milk not more than an average of 1,500,000 before or 100,000 after pasteurization.

It is of course essential that milk once pasteurized should be protected against contamination with pathogenic microbes. To this end the health of the workers in and about a pasteurizing plant is a proper matter for concern; careful oversight should be given also to those engaged in the work of distribution.

Bacillus Bulgaricus.—The name Bacillus bulgaricus is given to an organism isolated from milk in 1905 and made prominent by the work of Metchnikoff. The bacillus is a slender, gram-positive organism showing granular staining with methylene-blue. It produces a large amount of lactic acid in milk and shows marked

[1] Ayers: Bull. 342, U. S. Dept. of Agr., Sept., 1926.

ability to grow in highly acid media. It is similar to, if not identical with, the Boas-Oppler bacillus found in the gastric juice of patients suffering from carcinoma of the stomach.[1] Organisms of this group are found in large numbers in normal ensilage at an important stage of fermentation, and are believed to produce a large part of the acid found in silage fermentation.

Metchnikoff first suggested feeding cultures of this bacillus in cases of intestinal putrefaction, the underlying idea being that the large amount of acid produced by B. bulgaricus will restrain putrefactive bacilli. The benefits derived, however, in such cases are not due to the presence of the Bulgarian bacillus which does not seem to become readily naturalized in the intestinal tract, but to the consumption of the highly acid milk. Others maintain that the milk itself is rendered more easily assimilable, and that the body is consequently better nourished.

Whatever be the explanation of the bacterial therapy introduced by Metchnikoff, there seems no doubt that in certain cases the taking of milk soured by cultures of the Bulgarian bacillus is attended with success so far as improvement in intestinal conditions is concerned.

Bacillus acidophilus.—In recent years there has been a renewal of interest in sour milk therapy with particular reference to milk soured by pure cultures of Bacillus acidophilus (page 312). This organism is a normal inhabitant in the intestinal canal of infants and, to a lesser degree, of adults. It appears to be better adapted to maintenance in the intestine than B. bulgaricus, and hence, on *a priori* grounds, it may be expected to serve better in those cases in which the implantation of an aciduric flora is desired.

Rahe distinguishes four types of B. bulgaricus and four types of B. acidophilus[2] on the basis of variations in carbohydrate fermentations. B. bulgaricus strains all ferment dextrose, some ferment sucrose and raffinose, and none ferment maltose; all B. acidophilus strains ferment maltose as well as dextrose and vary in the fermentation of lactose, sucrose, and raffinose.

From the work of numerous investigators and particularly of Rettger and his colleagues[3] it appears that the types of bacteria

[1] Heinemann and Hefferan: Jour. Infect. Dis., 1909, 6, p. 304.

[2] Rahe: Jour. Bact., 1918, 3, p. 420.

[3] Rettger, L. F., and Cheplin, H. A.: "A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of Bacillus Acidophilus," New Haven, 1923, 135 pp.

developing in the alimentary tract may be significantly influenced by alterations in the chemical composition of the diet, by the ingestion of cultures of B. acidophilus with or without carbohydrates or by the ingestion of certain carbohydrates (lactose and dextrin) alone. There is some evidence to indicate that coincident with stimulation of the growth of B. acidophilus there is a suppression of the customary flora. In extreme cases the flora may come to be dominated not by B. acidophilus, but by the closely related organism, B. bifidus. When experimental subjects are fed cultures of B. bulgaricus with lactose or dextrin there follows a proliferation of B. acidophilus such as is obtained with the ingestion of the same quantity of carbohydrate alone.

The mechanism whereby a particular species of bacteria which normally is not a predominant type in the intestinal flora becomes implanted is undoubtedly highly complex. It was suggested that aciduric organisms may overgrow other types which are present in the contents of the intestine and on the mucous membranes because the acids which are formed in the course of their metabolic activity—in the presence of utilizable carbohydrates—are favorable to their growth and multiplication and unfavorable to the organisms whose optimal hydrogen ion concentration lies nearer or beyond neutrality. On the basis of P_H measurements upon the feces of white rats and of man Rettger and Cheplin inclined to doubt the validity of such an explanation. However, the work of Cannon and McNease[1] makes such an explanation seem not unreasonable. These authors report that concurrently with a change from the usual mixed, proteolytic flora to an aciduric flora in the intestine of the white rat there is a change in the hydrogen ion concentration of the contents of the cecum from P_H 7.0–7.1 to P_H 4.4 and of the contents of the colon from P_H 7.0–7.1 to P_H 6.4–6.8.

A number of authors have reported significantly favorable results obtained in the treatment of chronic constipation and other intestinal disorders with acidophilus milk. Thus, Gompertz and Vorhaus[2] report that a favorable influence was observed in the 200 cases of chronic constipation and 100 cases of diarrhea and mucous colitis which they studied. Cheplin, Fulmer and Barney,[3] Kopeloff,[4]

[1] Cannon and McNease: Jour. Infect. Dis., 1923, 32, p. 175.

[2] Gompertz and Vorhaus: Jour. Amer. Med. Assoc., 1923, 80, p. 90.

[3] Cheplin, Fulmer and Barney: Jour. Amer. Med. Assoc., 1923, 80, p. 1896.

[4] Kopeloff: Jour. Amer. Med. Assoc., 1923, 80, p. 602.

and others have similarly reported therapeutic success. Kopeloff found that sterile milk or pasteurized acidophilus milk do not relieve constipation, while milk containing viable B. acidophilus is effective. Hence he concludes that the effects produced are due to bacterial and not to physical or chemical influences. Viable B. acidophilus may be recovered from the feces during several months after acidophilus milk feeding.

The marketing of commercial preparations of organisms that produce lactic acid is plainly in need of control.[1] The approximate number of milk organisms of a suitable type and the absence of undesirable contaminants are important matters requiring expert supervision.

Milk Products.—*Butter.*—The share of bacteria in the processes involved in the manufacture of butter and cheese is important, although at present imperfectly understood. In the making of butter the proper ripening of the cream has long been recognized as essential to the production of a desirable flavor and aroma. Butter made from sweet cream is insipid, and lacks the pleasant taste of sour cream butter. On the other hand, if the ripening process be continued too long—for example, for six days instead of two—undesirable fermentations may set in, which injure the butter hopelessly. In other words, the products of some bacteria appear pleasant and aromatic to the average person, while the products of other bacteria are objectionable or even strongly offensive. Butter sometimes has a bitter, fishy, soapy, oily, or "turnip" taste, which completely destroys its palatability. Different species of bacteria have been reported as occurring in association with these various kinds of "bad" butter. Members of the B. coli group, for example, have been found in connection with the occurrence of the turnip taste. It is not yet certain to what extent the rancidity of butter is due to bacteria. The access of light and air certainly favors the development of the products—free acids—that give a rancid taste, but the influence of air seems to be indirect. That is, the presence of oxygen favors the multiplication of those aërobic bacteria which are able to split up the butter-fats. The action of light seems to be one of direct oxidation of the fatty acids. The becoming rancid of oleomargarine has been ascribed to the activity of two special kinds of bacteria.

[1] W. James: Jour. Amer. Med. Assoc., 1927, 89, p. 89.

When the manufacture of butter is carried on in a dairy or creamery under ordinary hit-or-miss conditions, it is largely chance that determines what kinds of bacteria take part in the ripening process. Some dairies happen to contain varieties of bacteria which have become naturally domesticated in the surroundings and produce palatable compounds, while others are infested with species that impart a bitter or tainted flavor to the ripened cream. For these reasons many attempts have been made to control the ripening process. Storch in Denmark, Weigmann in Germany, and Conn and Russell in the United States have been foremost in applying to butter-making the use of pure cultures of favorable germs.

Before the use of pure cultures it had been the custom in many successful dairies to use a "starter," that is, a small quantity of cream that had shown the looked-for qualities in ripening. This was added to fresh cream, the obvious if unconscious purpose of this proceeding being to impregnate the fresh cream with the specific organism or organisms present in the starter. Examination of the "natural starters" has shown that they are mixtures of several different organisms, but that bacteria able to produce lactic acid greatly preponderate (95 per cent.). The next step toward putting butter-making on a scientific basis was to remove the uncertainty due to the use of mixed cultures, and different investigators soon isolated micro-organisms which were able, especially under carefully controlled conditions, to imbue butter with an agreeable aromatic taste and odor. Over a score of such artificial starters are now said to be in use in various parts of the world. At the present time inoculation with pure cultures after preliminary pasteurization of the cream is practised chiefly in Denmark; in the United States the procedure has not won very extensive acceptance, partly, it is said, because the public taste in this country demands a butter with a rather strong flavor.

The nature and interrelationship of the several cultures used for cream-ripening is not altogether clear. All seem to be lactic acid bacteria; some are streptococci, apparently identical with Streptococcus lacticus (Heinemann). The difference in ability to produce desirable flavor is in many cases undoubtedly a racial or varietal rather than a specific difference, organisms otherwise closely allied differing in this respect. It seems important in all cases to

control the conditions of ripening (*e. g.*, to maintain a temperature of 60° to 75° F.) if the best results with artificial starters are to be obtained. Especially advantageous is the preliminary pasteurization of the cream, which eliminates most of the bacteria that might interfere with the free development of the organism added in the starter. Clean utensils and surroundings are quite indispensable to the successful use of an artificial starter (pure cultures) and the output of a product of uniform quality. In short, the manufacture of butter is tending to become essentially a bacteriologic process dependent upon the action of particular bacterial species and upon the conditions that surround their growth.

Fig. 174.—Camembert cheese cut open to show the softening of the curd (Conn).

Cheese.—The bacteriology of cheese-making is, generally speaking, on a more indefinite footing than that of butter-making. Some of the flavors characteristic of the different varieties of cheese are without doubt due to bacteria and molds, but the precise nature of the species concerned, and the conditions of their action, are in most cases largely a matter of conjecture. The casein or curd of milk, when freshly precipitated, usually by the addition of rennet, is insoluble; but a process of ripening ensues during which it is converted into soluble bodies. The digestion of the casein is effected principally through the agency of bacteria, but possibly in part also by enzymes in the milk (galactase, Babcock and Russell). Two types of cheese, the "hard" and the "soft," are made from ripened curd,[1] the difference being primarily due to a difference in the

[1] The so-called cottage cheese and the cheese sold in the United States under the name of Neufchâtel are merely unripened curd.

mechanical treatment of the curd. The milk for the hard cheeses is curdled rapidly, and much of the whey is separated from the curd by heating, manipulation, and pressure. For soft cheeses the whey is never thoroughly drained from the curd. The difference in consistency brings about difference in rapidity of ripening and in amount of bacterial action. The soft cheeses (Brie, Camembert, Gorgonzola, *et al.*) are ready for the table much sooner than the others, and are also more perishable. The flavor and odor of these cheeses are often strong and sometimes offensive, as in the case of the classic Limburger. In the ripening of certain varieties of the soft cheeses, only bacteria are thought to be concerned; in others, molds growing on the exterior contribute to the ripening (Brie, Camembert), and in still others molds permeate the whole curd, and are, perhaps, the main agents (Roquefort, Gorgonzola, Stilton). Some advance has been made in determining the species of micro-organisms that take part in the production of certain cheeses. The ripening of Camembert cheese, for example, has been carefully studied by Conn and his collaborators,[1] who conclude that two kinds of mold are necessary for the proper ripening and the development of the characteristic flavor (Figs. 175, 176). One of these is a species of white mold (Penicillium candidum?) closely allied to the common blue-green mold (Penicillium glaucum). This mold is not found in ordinary milk—in fact, for a long time one of the main obstacles to the manufacture of Camembert cheese in the United States was the failure to obtain and control this organism.

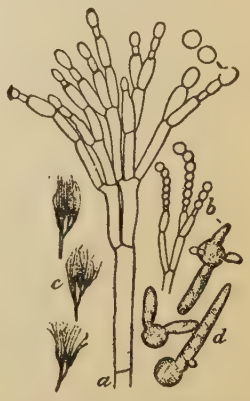


Fig. 175.—Penicillium of Camembert cheese, showing method of forming spores (Thom).

The texture of Camembert cheese seems to be due primarily to the growth of the species in question. Other species of Penicillium either fail to soften the curd characteristically or else impart to it an unpleasant bitter taste. The Camembert Penicillium (Fig. 175) forms a felted mass on the surface, penetrating perhaps $\frac{1}{16}$ of an inch into the curd. The spores of the mold usually ripen during the third week, and no further change takes

[1] Conn *et al.*: Bull. 35, Storrs Agr. Expt. Station, 1905.

place. "A cheese ripened by this mold alone is white, soft, creamy, and entirely palatable, but is wanting in color, and completely lacks the peculiar flavor for which Camembert cheese is sought in the markets." The Camembert *flavor* is due mostly, perhaps wholly, to the well-known and widely distributed mold or yeast-like organism, Oïdium lactis (Fig. 176). Much seems to depend upon maintaining a proper balance between the Penicillium and the Oïdium. A final conclusion as to whether or not Oïdium lactis alone produces the flavor will depend upon an exhaustive test of those bacteria so constantly associated with it.

Other cheeses derive their characteristic qualities from the proportion of various micro-organisms. In certain Swiss and Belgian soft cheeses the principal share in ripening is attributed to Oïdium lactis (Freudenreich and Marchal). The famous Roquefort

Fig. 176.—Oïdium lactis colony on gelatin (Conn).

cheese owes its characteristics, at least in part, to a Penicillium (P. glaucum?) which grows at the low temperature prevailing in the limestone caverns common in that district in France which gives its name to the cheese. Various kinds of Penicillium, perhaps all to be regarded as varieties of Penicillium glaucum, are particularly active in cheese ripening. They not only exert a proteolytic action themselves, but by their destruction and alteration of the acid products facilitate the action of the peptonizing bacteria. They also influence the flavor of the cheese. The sharp taste of such varieties as the English Stilton, the French Roquefort, and the Italian Gorgonzola and Parmesan cheeses is due to the products of these molds. The hard cheeses (Cheddar, Swiss, American, Edam, and others) are thought to be ripened exclusively by bacteria without intervention of molds. Edam cheese is made with slimy

whey as an artificial starter; this contains a streptococcus (Str. hollandicus).

The number of bacteria present depends to a large extent upon the age of the cheese. Both hard and soft cheese may contain enormous numbers, especially in the first days after preparation. Cheddar cheese, for example, has been found to contain as many as 635,000,000 bacteria per gram. After an apparently somewhat variable period of increase, a diminution sets in, and the numbers decrease at first rapidly, then more slowly. In old cheese as few as 1400 germs per gram have been found. The increase of the first two weeks is thought to be due principally to the lactic acid bacteria. At first, however, this group of bacteria shows a slight decrease, owing apparently to the fact that the peptonizing bacteria then have the upper hand (Russell and Weinzirl). Some difference of opinion has existed regarding the relative share of the peptonizing and lactic acid bacteria in the normal ripening process. The view especially advocated by E. von Freudenreich that the lactic acid bacteria are primarily concerned in the ripening of cheese has met with some opposition. Many investigators believe that the peptonization of the curd by aërobic, casein-dissolving bacilli of the Bacillus subtilis group—the Tyrothrix bacteria of Duclaux—or by casease-producing cocci (Weigmann) is to be regarded as the essential feature of the ripening of cheese. On the other hand, most investigators are agreed that the acid reaction brought about by the lactic acid bacteria is requisite to a normal ripening. Were it not for the restraining influence of the acid, the peptonizing bacteria would carry on their activities to the stage of putrefaction.

The ripening of cheese may, therefore, be considered as a process in which different groups of bacteria participate, one species after another gaining the upper hand (metabiosis). The lactic fermentation organisms are particularly important, since various decomposition processes are checked by their products. It has been urged by some investigators (Rodella) that anaërobic bacilli are intimately connected with the ripening of many varieties of cheese, and there is some evidence for this view.

The use of pasteurized milk inoculated with pure cultures or selected mixed cultures of micro-organisms has in some cases given rise to a normal ripened product, but in others has been unsuccess-

ful. The complicated nature of cheese-ripening, which seems to depend upon the successive activity of different groups of bacteria as well as upon the presence at the right time of suitable aroma-producing species, renders the bacterial control of cheese manufacture particularly difficult.

Both butter and cheese are liable to "abnormal" ripenings or fermentations which impair the palatability and other marketable qualities. Bitterness has been attributed to the products of various micro-organisms, such as Oïdium lactis, Bacillus fluorescens liquefaciens, and others. Sometimes genuine putrefactive changes are observed. Hard cheese is not uncommonly spoiled by the growth of gas-producing bacteria, which cause the formation of numerous cavities in the substance of the cheese. The "spongy" or inflated cheeses usually acquire also an unpleasant flavor. The bacteria concerned belong to the lactic group (Russell), and are perhaps to be identified with Bacillus aërogenes. The formation of the cavities is best prevented by carrying on the ripening at a temperature so low that the gas-producing organisms do not thrive. "Tainted" and "bitter" cheeses, as well as cheeses spotted or patched with color (chromogenic bacteria), are also reported from time to time. The rusty spots on American Cheddar cheese are due to a chromogenic bacillus named Bacillus rudensis.[1] This organism seems to find especially favorable conditions for its growth in spring and early summer, and, although it does not seem to injure the taste of the cheeses on which it grows, it does affect their marketability. The remedy for the nauseous or unpalatable fermentations of cheese and butter lies in a more scrupulous attention to cleanliness in the surroundings of factory and dairy, and generally in a more rigorous bacteriologic control.

Infection from Butter and Cheese.—Pathogenic bacteria which occur in milk may, of course, sometimes find their way into the milk-products. Butter and cheese made from the milk of animals suffering from foot-and-mouth diseases are known to have produced infections (Ebstein,[2] Thiele[3]). Tubercle bacilli (of bovine origin?) have been found in butter by a number of observers. One of the most recent tabulations of such investigations[4] shows that in 727

[1] Harding, Rogers and Smith: Bull. No. 183, N. Y. Agr. Expt. Sta., 1900.
[2] Ebstein: Deut. med. Wchnschr., 1896, 22, pp. 129, 154.
[3] Thiele: Deut. Militärärztl. Ztschr., 1900, 29, p. 548.
[4] Lafar: "Handbuch der Technischen Mykologie," 2, p. 31.

samples tested (mainly the market butter in German cities) tubercle bacilli were found in 88 samples, or about 12 per cent. Tubercle bacilli have also been reported in the quick-ripening varieties of cheese by Rabinowitsch,[1] Harrison,[2] and others. As pointed out in the chapter on tuberculosis (p. 444), the significance of such findings for public health is quite problematic. It is by no means impossible for milk and milk products to become contaminated with tubercle bacilli of human origin. The discharge of tubercle bacilli by a tuberculous milkman, for example, in the act of sneezing opens up many chances for infection. It needs no argument to show that the employment of tuberculous persons in the dairy is fraught with more or less peril to the community.

Many experiments have been made to determine the longevity of the typhoid bacillus in butter and cheese. Careful experiments by Berry[3] showed a survival period for the typhoid bacillus in butter of at least $3\frac{1}{2}$ months, and an even greater longevity for certain bacilli of the paratyphoid group. Epidemiologic evidence of butter-borne infection is obviously difficult to secure. The relatively long interval ordinarily elapsing between the production and consumption of butter and cheese undoubtedly renders the danger of typhoid infection much less than in the case of milk. The life of the cholera spirillum in butter and cheese is much shorter than that of the typhoid bacillus, and the danger of contracting cholera from the use of these foods is probably so slight as to be negligible. It is possible for the various milk products, just like other foods, to be contaminated independently after manufacture, and precautions to prevent such chance contamination are especially necessary in the case of foods that, like these, are commonly eaten without cooking.

[1] Rabinowitsch: Ztschr. f. Unters. d. Nahrungs u. Genussm., 1900, 3, p. 801.
[2] Harrison: Landw. Jahr. d. Schweiz, 1909, 14, p. 317.
[3] Berry: Jour. Prevent. Med. 1927, 1, p. 429.

CHAPTER XXXV

BACTERIA AND THE NITROGEN CYCLE

The fact that the chemical element nitrogen enters into the composition of all living things raises a number of important problems. One of these relates to the available sources of nitrogen. Not all forms of nitrogen are capable of being built up directly into living matter. The complex nitrogen substances in the body after death are disintegrated by bacterial activity, and it is well known that ammonia (NH_3) is one of the products of this decomposition. This nitrogenous compound is not an available food for forms of animal life, although it is directly utilizable by some of the higher plants. Certain bacteria, however, can oxidize ammonia to nitrates. It is a familiar fact that all animals depend ultimately upon plants for their supply of nitrogen, and that the higher plants derive their nitrogen for the most part from nitrates in the soil. The conversion of ammonia into nitrate by bacterial agency is, therefore, of peculiar interest, both practically and from a broad biologic point of view. Again, the atmosphere is a great reservoir of nitrogen, but in its elementary gaseous form nitrogen is wholly inert and useless for all the higher forms of life. In recent years it has been found that certain micro-organisms possess the singular ability of fixing free nitrogen. The supply of available nitrogen is, therefore, practically inexhaustible and to a considerable extent under control.

The relation of bacteria to nitrogen compounds may conveniently be considered under three heads: (*a*) Nitrogen-fixation, (*b*) Nitrification, (*c*) Denitrification.

(a) **Nitrogen-fixation.**—It is a striking fact that the store of nitrogen in ordinary uncultivated soil rich in vegetable matter increases naturally without human interference. Exact analyses have shown that the increase is due to the annexation of atmospheric nitrogen; the agency of micro-organisms in this process is attested by the fact that heated soil fails to show any nitrogen assimilation.

Nitrogen-fixation by Soil Bacteria.—Winogradsky was the first to demonstrate the share of a particular micro-organism in this process. Clostridium pastorianum (Winogradsky, 1895[1]) is a spore-forming anaërobe very closely resembling the butyric acid bacilli morphologically, but differing from them in its inability to ferment lactose and some other substances. When grown in a nitrogen-free solution of mineral salts and dextrose, the total nitrogen assimilation in twenty days may amount to as much as 53.6 mg. per liter. Winogradsky did not find any other organism capable of fixing free nitrogen, but more recently Beijerinck[2] (1901) has discovered a group of large aërobic bacteria which also possess this property. The name *Azotobacter* has been bestowed upon this genus, and several species have been described. One of the two species first described by Beijerinck (A. agile) is decidedly more motile than the other (A. chroöcoccum), and there are other slight differences (Beijerinck). A rather complicated symbiotic relation is believed by Beijerinck to subsist between Azotobacter and Clostridium or Radiobacter, but his view has not been generally accepted. Löhnis and Westermann[3] have compared a large number of strains from different sources. The cells of Azotobacter are round or oval bodies about 4 to 6 μ in diameter (Fig. 177); they are motile and possess rather short flagella; spore formation has not been observed; coccus forms, filaments, and pear-shaped involution forms are not uncommon; luxuriant growth does not occur on the ordinary culture-media. The amount of nitrogen assimilated is dependent, within certain limits, upon the amount of carbohydrate available, one experiment showing that while a solution containing 1 gm. of glucose gave an increase of but 7.4 mg. of nitrogen, a similar solution containing 12 gm. of glucose gave an increase of 127.9 mg. (Gerlach

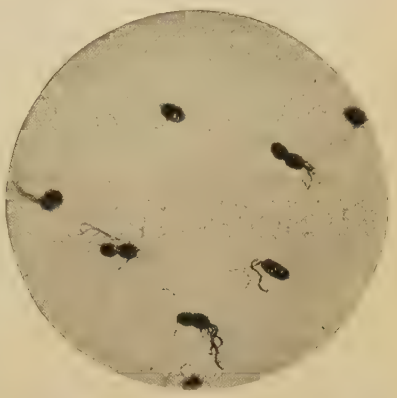


Fig. 177.—Azotobacter agile. × 1000 (Zettnow).

[1] Winogradsky: Arch. d. sci. biol., 1895, 3, p. 297.
[2] Beijerinck: Centralbl. f. Bakt., II, 1901, 7, p. 561.
[3] Löhnis and Westermann: Centralbl. f. Bakt., II, 1909, 22, p. 234.

and Vogel[1]). According to Stoklasa[2] the respiration processes of these organisms are wonderfully active; 1 gm. of bacterial substance (reckoned as dry substance) exhaling in twenty-four hours as much as 1.27 gr. of CO_2. This same author has observed the evolution of hydrogen in pure cultures of Azotobacter. Mannit solutions (*e. g.*, 1000 c.c. tap-water, 20 gr. mannit, 0.5 gr. di-potassium phosphate) and mannit-agar have proved the most satisfactory media for cultivating this micro-organism. Upon solid media the growth often has a black, brown, or yellow tinge.

It has been shown that certain other species of bacteria are able to assimilate free nitrogen. Among them may be mentioned B. mesentericus, B. pyocyaneus, B. prodigiosus, B. asterosporus, and the spore-forming aërobe, B. danicus (Löhnis and Westermann, *loc. cit.*). Certain of the common molds, such as Aspergillus niger and Penicillium glaucum, are claimed by several investigators to be capable of nitrogen assimilation, but the point is still in dispute. As respects algæ and the higher plants, independent nitrogen assimilation, although often affirmed, is not proved, many cases of apparent assimilation by these organisms being due to their symbiosis with some species of Azotobacter. Attempts to increase the yield of cultivated fields by inoculation with certain bacteria alleged to possess the power of nitrogen assimilation (B. ellenbachensis α = B. megatherium?; sold under the trade name of alinit) have not been generally successful, and the results of similar experiments with Azotobacter have also been negative. Azotobacter and Clostridium, as a matter of fact, seem to be universally distributed. It is, hence, more important to modify favorably the factors that influence the activity of these bacteria, such as the physical or chemical condition of the soil, than to add more bacteria to soil in which they are already present. These conditions have been sometimes summed up under the name of soil-climate (Bodenklima), and are being made the subject of study by many investigators. It is considered that the favorable modification of the soil-climate for the free nitrogen fixing organisms is one of the most important tasks before scientific agriculture. It has been shown already that certain carbohydrates favor greatly the nitrogen enrichment of soil to which they are added. Dextrose, saccharose, and probably also the carbo-

[1] Gerlach and Vogel: Centralbl. f. Bakt., 1902, II, 8, p. 669; 9, p. 817; 1903, 10, p. 636.

[2] Stoklasa: Centralbl. f. Bakt., II, 1908, 21, p. 506.

hydrates present in dried grain stalks (straw) are among the substances which increase the nitrogen assimilation of free living bacteria. Phosphates also exert a favorable influence. Potassium sulfate and especially potassium chlorid, on the other hand, are distinctly detrimental to the nitrogen-fixing activity. It is probable also that other bacteria present in the soil affect the fixation of nitrogen either through the action of their products or through direct competition for food material.

Fig. 178. —Root nodules of Lupinus luteus (Mayer).

Nitrogen-fixation by Nodule Bacteria.— One phase of nitrogen assimilation that has attracted universal attention, and that has long been familiar in certain of its practical aspects, is the accumulation of nitrogen by leguminous plants. One of the basic facts of agriculture is that while certain crops, notably the grains, exhaust the nitrogen in the soil, others, such as the clovers, peas, beans, and lupines, not only do not diminish the nitrogen-content, but augment it, so that their growth tends to enrich soil impoverished by other plants. Exact experiments have shown that a crop of crimson clover is able to add more than 200 pounds of nitrogen per acre. A long step toward the explanation of this remarkable fact was made by Hellriegel and Wilfarth in 1886.[1] These observers showed that the tubercles or nodules[2] (Fig. 178), long known to occur on the roots of the common Leguminosæ, and shown by Frank[3] (1879) to be absent from plants grown in sterilized soil, were not merely reserve storehouses for protein substances, but stood in active causal connection with the assimilation of free nitrogen. The nodules themselves are largely filled with rod-shaped organisms, which were discovered by

[1] Hellriegel and Wilfarth: Centralbl. f. Bakt., 1887, 1, p. 133 (Rev.).

[2] The root-tubercles were described by Malpighi in 1687 (Opera omnia, Leyden), but first recognized to be normal, not pathologic, structures by Treviranus in 1853 (Bot. Zeit., 11, p. 393)

[3] Frank: Bot. Zeit., 1879, 37, p. 377.

Woronin[1] in 1866, but whose bacterial nature was not definitely established until Beijerinck[2] grew them (1888) on an artificial medium composed of a decoction of pea leaves, gelatin (7 per cent.), asparagin (0.25 per cent.) and saccharose (0.5 per cent.).

The nodule-bacteria (Bacillus or Rhizobium radicicola) when of full size are rather large rods about 1 μ wide and 4 to 5 μ long; they are actively motile, strongly aërobic, and form no spores. On nutrient gelatin small, viscous, non-liquefying colonies are formed; their appearance has been compared to that of fat-droplets. Inside of the nodules the bacteria become metamorphosed into considerably larger branching structures, the so-called bacteroids. Bacteroids may also develop in cultures; they appear with great constancy in media rich in carbohydrates and certain organic acids (Fig. 179). Different species of Leguminosæ harbor bacteroids that are often strikingly different in size and shape. Functional differences also exist; bacteroids that are very active or "virulent" when in association with one species may refuse to form nodules on the roots of a closely allied species. Such facts have been thought by some observers to indicate the existence of a large number of more or less independent varieties of nodule bacteria. Others would restrict classification to two or more groups, but thus far there has not been general agreement upon the features characterizing such groups. Certain investigators have succeeded in transforming apparently independent varieties into one another, thus proving their essential identity. On the whole, it must be regarded as uncertain how far the nodule bacteria associated with different species of Leguminosæ are distinct, but there are probably at least two groups.

Fig. 179.—Development of root bacteria: *a*, root-bacteroids; *b*-*d*, forms in nodules of Vicia sativa (Beijerinck).

The view that the relation between nodule bacteria and host-plants is from the start one of true symbiosis in which both organ-

[1] Woronin: Bot. Zeit., 1866, 24, p. 329.
[2] Beijerinck: Bot. Zeit., 1888, 46, p. 725.

isms uniformly derive benefit from the association has lost ground in the face of recent researches. So far from welcoming the advent of B. radicicola to its tissues, the host-plant offers a determined resistance. The root-hairs constitute the usual portal of entry, and a very definite tissue reaction is produced at the point of invasion. Decided differences in the "virulence" of the bacteria are noticed (Hiltner[1]). Some bacteria are not able to effect an entrance at all; others enter and provoke a reaction in the tissues which leads to advantageous nodule formation; and still others injure the host-plant. In brief, the bacteria behave toward the plant, at least in the beginning, like true parasites against which the plant strives to protect itself with all possible means of defense. Eventually a state of equilibrium or kind of armed truce is brought about, in which both bacterium and plant benefit by the association.

The size, number, and activity of the nodules depend upon the qualities of the invading bacteria and upon the resisting powers of the host. As an example of the relation between plant and bacterium may be mentioned the action of saltpeter (potassium nitrate) in preventing nodule formation. The addition of a small quantity of this salt to a water culture of a nodule-forming plant suffices to abolish nodule formation altogether. According to Hiltner, the effect of saltpeter is due less to any strengthening of the plant's resistance than to its direct influence upon the nutrition of the bacteria. It may, however, increase the resistance of the plant by lessening its "nitrogen hunger." Inoculation experiments have brought out the interesting fact that the bacteria obtained from active nodules confer upon the plant immunity toward bacteria of the same virulence or of lower virulence; only those bacteria possessing a higher virulence than the ones in the nodules are able to penetrate the roots. There is thus a natural adjustment between the resistance of the plant and the invasive power of the micro-organism.

The question how the possession of root-nodules enables the leguminous plant to transfer free nitrogen from the atmosphere to its tissues has received various answers. Frank, one of the earliest investigators in this field, came to the conclusion that nitrogen assimilation was a property of all green plants, and that the nodule bacteria acted simply as a "stimulus" to provoke its more intense manifestation. The fact, however, that no direct nitrogen-fixation

[1] Hiltner: Arb. a. d. k. Gesund., 1906, 1, p. 175.

by green plants could be proved weighed heavily against this view, which, indeed, was soon abandoned by its author. Subsequent researches have plainly shown that the accumulation of nitrogen takes place first of all inside the nodule, and that the bacteroids are the seat of the active processes. B. radicicola is able of itself in pure culture to fix atmospheric nitrogen, but in much smaller quantity than in the legumes. Granting that the bacteroids gather nitrogen, how is it transferred to the plant? It has been assumed that the bacteroids are absorbed bodily, but against this supposition stands the disproportionately small quantity of nitrogen in the bacteroids of all the nodules of the plant as compared with the total nitrogen gain of the plant. Nobbe and Hiltner[1] cite an instance in which a plant had taken 1 gm. of nitrogen from the air, although all the nodules on its roots weighed only 300 mg. Still another conception, based upon definite chemical observation, has been recently received with much favor. This is the view that certain compounds formed by the bacteroidal protoplasm are soluble and diffusible through the cell wall, and that these, passing out from the bacteroids, are taken up by the host-plant.

Mazé based an interesting theory of nitrogen utilization upon the great viscosity of cultures of Bacillus radicicola. The viscosity is due to the presence of a gum which is a slightly modified portion of the capsule or outer portion of the cell wall of the organism. It was Mazé's assumption that this gum or mucus was a nitrogenous compound, and that the simultaneous production of this substance and the fixation of nitrogen in solution were proof of its connection with the latter process. Buchanan,[2] however, has shown that the gum contains no combined nitrogen, but is a carbohydrate substance closely related to the dextroses produced by other groups of bacteria. Its connection with the fixation of nitrogen by the legume organism is, therefore, improbable.

Broadly considered, therefore, the process of nitrogen-fixation by leguminous plants consists, first, in the penetration of the root tissues by certain bacteria which establish themselves there in a sort of half-parasitical, half-symbiotic relation; second, in the accumulation of nitrogenous substances by the bacteria under the influence of the abundant carbohydrate food-supply available in the plant

[1] Nobbe and Hiltner: Centralbl. f. Bakt., 1900, II., 6, p. 449.
[2] Buchanan: Centralbl. f. Bakt., II., 1909, 22, p. 371.

tissues, the nitrogen used in the constructive process being derived from the atmosphere; and, finally, in the appropriation by the plant of the nitrogenous compounds contained within or diffusing out from the nutritionally and structurally modified bacteria (bacteroids). The conditions under which the plant extracts nitrogenous substances from the bacteroids is yet unexplained, but there are facts that seem to connect the phenomenon with a state of "nitrogen hunger," or lack of other sources of nitrogen supply, such as the nitrates of the soil or the nitrogenous stores in seeds. The "virulence" or invasive power of the micro-organism may be connected with a similar condition in the plant.

Many attempts have been made to utilize the enrichment of the soil that results from nitrogen-fixation. The custom of green manuring—that is, of plowing under leguminous crops—which has been long practised empirically, has won much wider extension as a consequence of the establishment of its underlying principles. The discovery that certain soils on which leguminous plants grow feebly could be made to yield much more luxuriantly by soil inoculation has given rise to a train of experiments having for their purpose the perfecting of the relations between bacteria and plants. In some of the earliest ventures, soil obtained from fields where the proper bacteria were present in abundance was used for infecting other fields where leguminous plants failed to grow or developed poorly. The results were in some cases brilliantly successful. Following up this success, pure cultures of nodule bacteria were employed experimentally and were marketed on a large scale under names such as "nitragin" or "nitro-culture." Impregnation of the soil with these pure cultures has by no means been uniformly successful, although in some cases it has given unquestionably good results. Inoculation of seeds with pure cultures of the nodule bacteria has led in many hands to somewhat better results than inoculation of soil, but cannot be unreservedly depended upon. One of the reasons for the rather frequent failures reported is the lack of the requisite technical skill and judgment in the application of pure cultures; another is that the presence or absence of proper nodule bacteria is only one of many factors that determine the prosperity of leguminous plants; and still another is that the "virulence" of the culture is at present not wholly susceptible of control. The upshot of practical experience in the United States has been

that the use of pure cultures is attended with much uncertainty, and that "the simplest and surest and most economical method of inoculation is by means of well-infected natural soil, collected where the proper bacteria are found in abundance (as shown by the tubercles on the roots of the plants) and scattered over the field to be seeded at the rate of one hundred pounds or more per acre."[1] It can hardly be doubted, however, that greater precision will eventually be introduced into the practice of soil inoculation, and that the use of pure cultures is likely in the future to become more important than at present.

As already stated, all the members of the family of leguminous plants are endowed with nodules. In this respect they stand almost but not quite alone, since similar nodules have been observed on other plants, notably the alders. The alder nodules apparently sustain the same physiologic relation to the plant as do the leguminous nodules, and enable the alder to gather nitrogen from the atmosphere. There has been much dispute as to the nature of the organisms present in the alder nodule, but several investigators agree in regarding them as very much like the bacteroids, although hyphomycetal characters are rather more pronounced.

A peculiar mycelial growth, known as mycorrhiza, was observed first in association with the roots of orchids, and has since been found in and upon the roots of many other plants; probably it occurs either constantly or occasionally on the roots of the majority of the higher plants. A number of different molds, some of them belonging to common species, are able to develop this singular structure. While there are many points about the physiologic significance and development of mycorrhiza that are far from being elucidated, it seems to be definitely established that at least the endotrophic mycorrhiza is able to fix free nitrogen. So far as determined, the protoplasmic changes within the hyphæ are like those in the bacteroids, and the whole physiologic process of nitrogen-fixation and plant-absorption is probably the same. Possibly the ectotrophic mycorrhiza has the same function, but this seems doubtful.

(*b*) **Nitrification.**—The complex nitrogen compounds that are among the most important constituents of the body-substance of all forms of life furnish a source of energy not overlooked by bacteria. As soon as an animal or plant dies and the influences that restrain

[1] Illinois Agr. Exp. Sta. Cir., No. 86.

bacterial activity vanish, a breaking-down process, due to bacteria, sets in, which ends in producing substances that are chemically of simple structure. Practically nothing is known about the earliest stages of protein decomposition, not only because the constitution of the protein molecule is largely conjectural, but because the process is almost hopelessly complicated by a variety of modifying influences. Eventually out of the seething caldron of molecular disintegration emerge such relatively simple bodies as the organic acids and amins, mercaptan, sulfureted hydrogen, carbon dioxid, and ammonia. Some of these substances are susceptible of further decomposition. Ammonia, for instance, which is produced abundantly from nitrogen-containing compounds, may be oxidized to nitrites, and the nitrites oxidized in turn to nitrates. This process has received the name of **nitrification.** Apart from its theoretical interest, nitrification is of great agricultural importance, since it is the means by which the ammonia produced from decaying vegetable matter and from manures is converted into a form utilizable by the growing plant.

Like other processes of oxidation, nitrification was long believed to be due simply to the action of atmospheric oxygen, or in some cases to that of ozone. The share of living micro-organisms in the process was first definitely foreshadowed by Pasteur's discoveries concerning acetic fermentation, another oxidation process. Pasteur himself clearly expressed his conviction concerning the essential nature of nitrification in 1862, but his suggestions were not acted upon until Schloesing and Müntz took up the question in 1877.[1] These investigators carried on a series of extensive researches which showed convincingly that living organisms were at the bottom of the phenomenon. Chemical substances like chloroform, that check or interfere with microbes, prevent the process of nitrification: heating to temperatures that effect sterilization likewise abolishes it; on the other hand, temperatures that favor the activity of bacteria and their allies promote nitrification. While thus successful in demonstrating that micro-organisms were answerable for the occurrence of the natural process of nitrification, Schloesing and Müntz were not able to fix the responsibility upon any particular species. In fact, for some time it remained doubtful whether many different kinds of bacteria might not possess the ability to oxidize ammonia

[1] Schloesing and Müntz: Compt. rend. acad. d. sci., 1877, 84, p. 301; 85, p. 1018; 1878, 86, p. 892; 1879, 89, pp. 891, 1074.

to nitrates. The isolation of an unmistakable nitrifying organism in pure culture was first accomplished in 1888 by Winogradsky.[1]

It appeared from Winogradsky's researches that the main reason why previous investigators had been baffled in their effort to isolate a nitrifying organism was that they placed too implicit confidence upon the ordinary gelatin culture-medium. The nitrifying organism does not grow upon nutrient gelatin, and while readily cultivable upon solutions containing simple ammonium salts, is distinctly inhibited by the presence of organic matter. At first it was supposed that the whole process of nitrification from ammonia to nitrates could be completed by a single species, but it was later found that the chemical division of the process into two stages, first, the oxidation of ammonia to nitrites, and then that of nitrites to nitrates, corresponded to the physiologic activity of two different groups of micro-organisms.

Nitrification may be readily provoked in solutions of certain mineral salts by the addition of a small amount of ordinary cultivated soil. Provided the nitrifying organism is present and organic matter is absent, the process is not interfered with by the presence of foreign micro-organisms. A simple nitrifiable solution used by Winogradsky in his researches has the following composition:

Ammonium sulfate	1 gm.
Potassium phosphate	1 "
Well-water	1000 c.c.
Basic carbonate of magnesia in excess.	

The fact that the transfer of a small quantity of material from a solution that had undergone nitrification sometimes induced complete nitrification in a fresh solution, sometimes carried the process only to the formation of nitrites, and sometimes failed altogether, was at first explained by assuming that the nitrifying organism had become weakened; but the discovery that the production of nitrites and that of nitrates were independent processes, due to the activity of distinct organisms, finally afforded the true explanation. Nitrites cannot be formed unless the nitrite-forming organism is present: if the nitrite-former alone is present, the process stops midway; if the nitrate-former alone, the first step cannot be taken.

The nitrite-forming organism discovered by Winogradsky has received the name *Nitrosomonas*. Unlike the common bacteria,

[1] By the dilution method.

it has a definite life-cycle. When a vigorous culture is inoculated into a suitable mineral solution, a strong nitrite reaction develops by the end of four or five days. At this time scattered cells are few, most of the organisms being gathered in compact zoöglea-like masses in the sediment at the bottom of the flask. Staining with a weak solution of iodin in potassium iodid brings out the structure of these masses most satisfactorily (Winogradsky). Some days later (seven to ten) these aggregates are found to have resolved themselves into separate ellipsoid cells, which bear a flagellum on one end and swim actively about through the fluid. After twenty-four to forty-eight hours the swarming cells become quiescent and sink again to the bottom, where they remain singly or joined in small groups. Variations from this process are not uncommon, but Winogradsky regards the development just described as typical. The zoöglea cells are thought by Winogradsky to represent a resting stage; they are somewhat more resistant to drying than the free cells.

The description given above holds good only for the nitrite-forming organism found in western Europe (Zürich, Gennevilliers). The nitrite-formers found in St. Petersburg (no swarming stage observed), in Java (swarming stage present), and in Quito, South America (no swarming stage certainly observed), differ morphologically from the Nitrosomonas of western Europe and from one another. The peculiarities are constant and are maintained in cultures for a considerable period, but it is yet uncertain whether the differences correspond to true specific differences or are merely the expression of differences in local conditions. No two forms of the nitrite-producing organisms have ever been found in one locality. The cultivation of Nitrosomonas on solid culture-media was first achieved by Winogradsky by the use of silicic acid jelly ("water glass").[1] The organism has also been grown in the presence of suitable ammonia salts on washed and purified agar by Beijerinck,[2] and on gypsum magnesium carbonate plates and on disks of filter-paper by Omelianski.[3] On the silicic acid medium the colonies are quite

[1] The successful preparation of this medium requires a considerable degree of experience. Details are given in a paper by Omelianski (Centralbl. f. Bakt., Abt. II, 1899, 5, p. 537). See also Lafar's "Handb. d. techn. Mykologie," 3, pp. 155–8; and Stevens and Temple: Centralbl. f. Bakt., II., 1908, 21, p. 84.

[2] Beijerinck: Centralbl. f. Bakt., II, 1896, 2, p. 698.

[3] Omelianski: Centralbl. f. Bakt., II, 1899, 5, p. 652; II, 1902, 8, p. 785.

characteristic, though they always remain small and are best studied with a magnification of about 100 diameters. At first colorless, they soon become dead brown and opaque, the surface colonies being rounded, the deeper colonies more irregular in outline. Later the colonies become granulated and translucent. This change from opaque to translucent colonies corresponds to a morphologic transformation from zoögleæ to free cells.

A highly remarkable physiologic peculiarity of the nitrite-forming organism is its ability to grow normally and produce active oxidation in a medium devoid of all trace of organic substance. The formation of nitrite has been shown by Winogradsky to depend upon the presence of carbon dioxid either free or in loose combination, and is accompanied by the accumulation of organically bound carbon, the amount of carbon assimilated bearing a definite proportion to the nitrogen oxidized. There seems no escape from the conclusion that Nitrosomonas, a colorless organism, and one therefore unable to utilize the energy of light rays, assimilates carbon dioxid by virtue of the energy obtained by the oxidation of ammonia. Under some conditions carbon dioxid appears to be the only available source of carbon, since organic substances, like glucose and peptone, not only do not favor growth, but exercise an inhibitory influence upon the nitrite-forming organism, and may even check it altogether. Corresponding to this inability to utilize the carbon of organic compounds is its inability to effect the oxidation of organic nitrogen. When grown in pure cultures in solutions, in the presence of nitrogenous substances like urea, asparagin, and egg-albumen, Nitrosomonas remains impotent, and no trace of either nitrite or ammonia formation is found after the lapse of months. Not even the amins, which are so closely related chemically to ammonia, can be attacked.

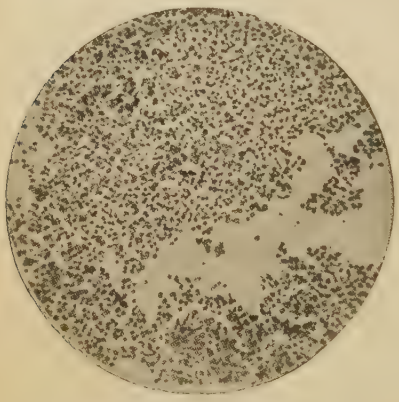


Fig. 180.—Nitrate-former from St. Petersburg nitrate. Agar. Fuchsin. × 1000 (Lafar).

As already stated, the oxidation of nitrites to nitrates is effected by another organism, which differs in some important respects from the nitrit former. It is much smaller, is provided with a capsule, and stains with difficulty, the pointed ends not staining as deeply as the middle (Fig. 180). No "swarming stage" has yet been observed. Growth occurs both in a nitrite solution[1] and on nitrite agar. It is, however, extraordinarily slow; after two weeks the round or oval colonies in the depth of the agar have attained a diameter of only about 30 μ to 50 μ. The colonies that come to the surface are round, colorless droplets, and may reach a size three or four times as great as those in the depths. In the fluid medium neither turbidity nor sediment makes its appearance, and usually no sign of growth can be observed. By repeated enrichment of a culture with nitrite, however, an opalescent veil forms slowly over the walls and bottom of the flask, and microscopic examination shows this film to be composed of spindle-shaped rods that stain with difficulty.

The physiology of the nitrate-former has been less completely worked out than that of the nitrite-producing organism. It seems probable, in view of experiments already made by Winogradsky and Omelianski and by Gärtner, that, like Nitrosomonas, the nitrate-former can obtain its carbon supply only from carbon dioxid. The nitrate-former is less sensitive toward the presence of organic substances than the nitrite-former, although both its development and, to a less degree, the production of nitrates are interfered with to some extent. On the other hand, ammonia exercises a remarkably harmful influence upon the nitrate-former, restraining its development more powerfully than the strongest antiseptic (Winogradsky). Nitrates, even in considerable quantities, do not hinder nitrate formation.

The fact that under varied natural conditions the process of complete nitrification goes on steadily and quite rapidly is not in reality out of accord with the singular physiologic qualities and limitations

[1] Sodium nitrite (natr. nitros-puriss, Merck) ..... 1.0 gr.
Potass. phosphate ..... 0.5 "
Magnes. sulfate ..... 0.3 "
Soda (water-free) ..... 1.0 "
Sodium chlorid ..... 0.5 "
Ferrous sulfate ..... 0.4 "
Distilled water ..... 1 liter

of the nitrifying organisms. Although neither nitrite- nor nitrate-forming organisms are able to generate ammonia from organic substances, they are constantly in association with myriads of bacteria in the soil and in water which do produce ammonia in abundance. Given ammonia as a starting-point, nitrite formation can occur, provided too much organic matter be not present, while after nitrite makes its appearance, the nitrate-former can pursue its activity. Ammonia seems to be injurious, especially to the development of the nitrate-former, less so to its oxidizing activity; however, both phases of the nitrifying process can go on simultaneously, provided living cells of the nitrate-forming organism are abundant at the time nitrite is produced. There is hence no real contradiction between the phenomena of nitrification observed under natural conditions and the characteristics of the nitrifying organisms exhibited in pure culture.

It should be especially noted that in soils the conditions of life for the nitrifying organisms are so different from those in solution that a direct comparison of metabolic activities is hardly possible. The presence of a multitude of other micro-organisms, and the difference in chemical and physical conditions where large amounts of sand and earth are in contact with the liquid medium, are factors that must influence powerfully the nitrifying processes. Stevens and Withers,[1] in fact, have shown that nitrification can proceed vigorously in the soil in the presence of large quantities of such organic matter as cottonseed meal, peptone, and cow manure.

(*c*) **Denitrification.**—Essentially different chemical and bacterial processes are quite commonly included under this head. Among these are a variety of reducing processes, such as the reduction of nitrates to nitrites and ammonia, the reduction of nitrates and nitrites to gaseous oxids of nitrogen (N_2O, NO), and the complete reduction of nitrates and nitrites with evolution of nitrogen gas ("true denitrification"). Other processes, such as the setting free of nitrogen in the course of protein decomposition and the constructive utilization of nitrate nitrogen, are quite far removed from the strictly reducing actions.

The ability to reduce nitrates to nitrites and ammonia is a widespread bacterial characteristic; Maassen,[2] who investigated the

[1] Stevens and Withers: Centralbl. f. Bakt., II., 1910, 27, p. 169.

[2] Maassen: Arb. a. d. k. Gesund., 1901, 18, p. 21.

reducing power of one hundred and nine kinds of bacteria, found eighty-five to possess this power. In reducing processes of this nature the presence of organic nitrogenous matter is necessary; on the other hand, glucose and other carbohydrates exert a hindering influence. It is not clear whether any physiologic significance should be attributed to the reduction of nitrates to nitrites, or whether the process is incidental and the reduction due simply to the chemical products of the bacteria. It is possible that, as in the case of the true denitrifying bacteria to be considered presently, the reduction is caused by the need of the cells for oxygen.

The formation of the oxids of nitrogen (N_2O and NO) is less commonly observed. It is not yet known to what extent this phenomenon is dependent upon conditions of growth, or how far it is a specific peculiarity of certain species. Among the more familiar species that can give rise to these gases under suitable conditions is B. pyocyaneus.

The "true" denitrifying bacteria, those that are able to reduce nitrates with the formation of free nitrogen as an end-product, are relatively few in number, but include such well-known species as B. coli, B. typhosus, B. fluorescens, and B. pyocyaneus. In nitrate broth (*cf.* p. 37) denitrifying species cause a foamy appearance which is quite characteristic, and is due to the liberated nitrogen. Light is thrown upon the physiology of the denitrifying bacteria by the circumstance that while they ordinarily grow under aërobic conditions, they can also thrive anaërobically, provided nitrite or nitrate be present. The inference seems plain that in these cases the reduction of nitrates is to be regarded as due to the respiratory needs of the micro-organism, which wrests the oxygen from its combination with nitrogen, setting free the latter as a gas. Jensen,[1] however, points out that denitrification must always be accompanied by oxidation processes. The same author has found that denitrifying bacteria produce considerably more peroxidase than other bacteria. The necessary conditions for denitrification are: (1) the presence of nitrates; (2) the presence of certain specific micro-organisms; (3) a considerable amount of readily assimilable organic substance; (4) limited access of oxygen.

[1] Jensen: Centralbl. f. Bakt., II, 1909, 22, p. 314.

The practical importance of soil denitrification for agriculture has been considerably exaggerated. Loss of nitrogen by this means can play an important part only if very large quantities of fresh manure are added to a soil exceedingly rich in nitrates or if manure and nitrates be added simultaneously.

CHAPTER XXXVI

BACTERIA IN THE ARTS AND INDUSTRIES

From its birth bacteriology has been more or less concerned with practical pursuits. As has been pointed out in the first chapter, the science owes its origin largely to the studies of Pasteur upon the phenomenon of fermentation; in particular, to his memorable researches upon the diseases of beer and wine. The further development of bacteriology has brought it unexpectedly into contact with a variety of industries and occupations outside of alcoholic fermentation, disclosing previously unseen opportunities for exploitation and utilization. The share of bacteria in advantageous or commercially valuable processes has so far been less thoroughly exploited than their agency in inciting disease, but the field already opened is a wide one. Much of the work that has been accomplished in this direction is special and technical; space forbids its consideration here except in its broader aspects. Some of the applications of bacteriology to agriculture have been touched on already in connection with the nitrogen cycle and with the rôle of bacteria in the dairy; but there are also several special industries which are in part or altogether bacterial processes, or which depend upon the proper application of bacterial methods.

Bacteria in Tanning.—Bacterial activity is involved at several points in the tanning process, the object of which is to treat the animal skin so that it shall offer the greatest possible resistance to decomposition without losing the qualities for which leather is valued. In the process of tanning, hides which have been previously protected against decomposition by drying or salting are freed from hair either by a carefully controlled decomposition, in which Proteus (p. 476) is said to be specially concerned, or by simple chemical treatment with sulfite of sodium or lime. After depilation, the hides are "drenched" or steeped in a liquor prepared according to various formulas that read like the therapeutic mixtures of the middle ages. Animal excrements, especially the droppings of hens, pigeons, and dogs, are a common ingredient; bran is also used

ordinarily in these mixtures. In the "bran drench" the essential feature seems to be that the starch of the bran is hydrolyzed and converted into sugar, and an acid fermentation occurs in which lactic acid is usually most abundant, although acetic, formic, and butyric acids are also found. The lactic acid is the active agent in removing the lime from the skin. Gas-forming bacteria are present in this fermentation, one of them having been described under the name of B. furfuri.[1] It is obvious that the heterogeneous character of the mixture introduces many uncertainties into the process. Attempts have been made to put the treatment on a more systematic basis. A distinction has been made between "bating" and "puring," bird dung being used in the first process and dog dung in the puring of the lightest leather. In spite of the great disadvantages that attend the use of such substances, practical tanners assert that they give results with some skins, notably goat skins, that no other material does. Substitutes for the objectionable animal feces have been proposed and used with some success. Wood[2] has carried on extended researches on the kinds of bacteria concerned in "bating" and "puring." One organism, B. erodiens, has been used in pure culture with results said to be very satisfactory. (See Wood[2].) The bated hides are placed either in a tan-pit (the coarse kinds destined for sole-leather) or in bark liquor (the thinner skins). The souring of the bark liquor is considered in Europe to influence favorably the quality of the product, making the leather soft and supple. Tanners have sometimes seeded fresh bark liquor with old soured liquor in order to insure that the fermentation takes a proper course. It is uncertain whether the favorable effect is due primarily or wholly to the acid or whether other bacterial products are concerned. As might be expected, numerous micro-organisms of different sorts—bacteria, yeasts, and molds—occur in the bark liquor. Varying amounts of carbohydrates and nitrogenous compounds in bark liquors of different origin influence materially the souring process and determine to some extent the character of the product. American tanners for the most part avoid sour liquor. A beginning is being made in the study of bacterial action in the curing of animal skin and will

[1] Perhaps the same as B. gasoformans.

[2] Wood: Jour. Soc. Chem. Ind., 1894, p. 218; 1895, p. 449; 1898, pp. 856, 1010; 1899, pp. 117, 990; 1910, 29, p. 666.

doubtless lead to the control of the proteolytic bacteria that influence this process.[1]

The Curing of Tobacco.—The organic substances present in the leaves of the tobacco plant are exposed, at the death of the plant, to a variety of bacterial influences. The first stage in the treatment of the leaf, the drying in specially prepared rooms, is not, however, a bacterial process, the conspicuous changes that occur, such as the dissolving of the starch and the browning, being due to leaf enzymes. Certain maladies may overtake the leaf at this stage: some of these are caused by saprophytic fungi (*e. g.*, Botrytis); the so-called "pole-burn" has been attributed in part to putrefactive bacteria.

In the next stage the dried tobacco leaves are ripened by being heaped up in great piles which undergo heating and fermentation. The temperature in the interior of these masses may rise as high as 50° C., exceptionally as high as 61° (Suchsland). Among the more noteworthy chemical features of this fermentation is the loss of nicotin, which amounts to about 28 per cent.

The share taken by micro-organisms in the ripening process has been and still is a matter of dispute; some observers hold that the oxidizing enzymes of the tobacco leaf itself are responsible for the changes that occur in this stage. Since a desirable aroma and other qualities of a successful product are dependent upon the course of the fermentation process, it is a matter of no small importance to the tobacco industry that the nature of the process should become clearly understood as the first step toward efficient control. Bacteria of a great variety of kinds are naturally not lacking in the fermenting masses of tobacco leaves. Many attempts have been made to determine what kinds, if any, are of significance in the ripening process.

The majority of those investigators who have studied in the laboratory the ripening of tobacco after inoculation with pure cultures of bacteria have obtained a product that, on the whole, possesses a more satisfactory aroma than tobacco ripened under natural conditions. The bacteria found in tobacco-curing seem to belong for the most part to the Proteus, Subtilis, and Mycoides groups.

[1] McLaughlin and Rockwell: Jour. Amer. Leather Chemists Assoc., July, 1922; June, 1923; July, 1924; July, 1925.

Some investigators (Suchsland, A. Koch) have used for inoculation cultures of bacteria obtained by them from tobacco "of the finest quality." It is said that spraying tobacco leaves with these cultures has imparted highly satisfactory qualities to a grade of tobacco which, if allowed to ferment simply by the aid of native bacteria, would have been of inferior character. The nature of the culture employed in this case has not been divulged, and it seems to be a fact that the method of using such cultures has not won any wide acceptance in the tobacco industry.

The relatively high temperatures in the interior of the ripening masses undoubtedly favor the development of thermophilic varieties; Vernhout has reported the constant occurrence of a thermophilic bacterium, belonging to the potato-bacillus group, in fermenting leaves of Java tobacco.[1] Additional evidence in favor of some bacterial share in tobacco-curing is given by the existence of the practice of "petuning." This custom consists in sprinkling the unripened leaves with a liquid prepared in various ways, such as by infusing old tobacco, mixing water with molasses, rum, etc. The petuning liquid used on different plantations is different and is often prepared by exact formulas. Havana tobacco fermented in the United States does not develop the true "Havana flavor." There seems to be no doubt that petuning enhances the aroma and value of the product, and that in some manner the quality of the tobacco produced on different plantations is affected by the nature of the petuning liquid applied. Such a fluid may conceivably act either by stimulating the growth of desirable bacteria already present on the leaves, or by infecting the leaves with the varieties that impart aromatic qualities.

On the other hand, Loew[2] has expressed the opinion that the curing of tobacco is not in essence a bacterial process, but is due to the action of leaf enzymes (oxidase, peroxidase, katalase) which effect all the chief changes that are observed. According to Loew's view, bacterial intervention is not necessary to secure a desirable product.

Both enzymes and bacteria are perhaps concerned in the curing process, although the relative and absolute importance of the two factors is still uncertain. If bacteria grow at all upon the ferment-

[1] See Lafar: "Hand. d. techn. Mykologie," 5, p. 10.
[2] Loew: Report No. 59, U. S. Dept. of Agri., 1899.

ing tobacco leaves, there seems no escape from the conclusion that their products must permeate to some extent the ripened tobacco. Whether the bacterial influence preponderates over that of the enzymes, and whether certain species give a better flavor than others, cannot at present be positively asserted. Jensen[1] has reported experiments which cast doubt on the microbic nature of the tobacco fermentation. The process is not hindered by germicides such as mercuric chlorid, formalin, and chloroform; on the other hand, the characteristic qualities of the fermentation can be produced, at least in part, by heating the tobacco leaves at a temperature of 90° to 100° C. for ten minutes to two hours. The characteristic rye-bread odor of freshly fermented tobacco, for example, can be brought about by this procedure. It is true that the oxidizing enzymes described by Loew are destroyed by high temperatures like those used in Jensen's experiments, so that according to these results both leaf enzymes and bacteria would seem to be excluded as essential factors in tobacco curing.

The Preservation of Foods.—The fact that many valuable foods, both of animal and plant origin, are abundant at certain seasons of the year and in certain localities, and are lacking or scarce in others, has made it a matter of great importance to the human race to find some way of preserving such food-products against natural decomposition. Simple methods of food preservation have long been practised. One of these is drying. Fish, meat, and fruits may be exposed under suitable conditions to sun and air, and the consequent loss of moisture renders the organic substance unadapted for bacterial growth (p. 82). Meats are sometimes smoke-dried, a process which involves another process besides drying, namely, the impregnation of the meat with antiseptic substances, such as creosote, present in ordinary wood smoke. Neither smoking nor pickling, however, can be depended upon to free meat from any pathogenic bacteria it may originally contain. Tubercle bacilli in smoked meat and the bacilli of swine erysipelas in pickled pork, for example, have been observed to retain their vitality and virulence for long periods.

The addition of chemical substances that check or inhibit decomposition is a time-honored mode of food preservation. Common salt and sugar are frequently used for this purpose. Preserves to

[1] Jensen: Centralbl. f. Bakt., II., 1908, 21, p. 469.

which a large quantity of sugar has been added and meats pickled in brine are not adapted for bacterial growth because of their great avidity for water. A germ falling into a strong sugar or salt solution is unable to grow because the density of the solution is greater than that of the cell protoplasm of the germ and tends to extract water from it. "Condensed milk" owes its keeping qualities when exposed to the air to the large amount of sugar it contains. The acetic acid of the vinegar used in pickling is another well-known chemical preservative. Sometimes rather strongly germicidal chemical substances, such as salicylic acid, borax, boracic acid, formaldehyd, and sodium sulfite are employed as food preservatives. While these last-named compounds undoubtedly prevent the decomposition of food, there is evidence that they are likewise injurious to the health of the consumer.

In some few cases the access of bacteria to the decomposable substance may be partially interfered with so that decay or putrefaction is to some extent retarded. The shell of the hen's egg is so porous that bacteria can pass through after the egg is laid, and many attempts have been made to preserve eggs by giving them an impervious coat. The most successful of the substances experimented with is the commercial fluid known as "water-glass," a syrupy mixture of sodium and potassium silicate (1 part of water-glass to 10 of water); but success in preservation is only partial, since eggs have usually become contaminated with bacteria while still in the oviduct, and hence at the time they are laid contain a considerable number of germs. Eventually eggs treated with water-glass will decay.

Many fruits, such as apples and pears, can be kept for a long time from spoiling if the skins are carefully wiped and dried and if bruising is avoided. In other words, any measure that tends to make difficult the growth or ingress of bacteria is an aid toward preservation.

A familiar method of food preservation and one widely employed at the present day is storage at low temperatures. Bacterial multiplication is greatly checked by temperatures a few degrees above freezing, and ceases at or near the freezing-point. Refrigeration is consequently an effectual hindrance to decomposition.

Much food in the large cold-storage warehouses is maintained in a frozen condition, and its wholesomeness is in no degree impaired

throughout a considerable period, although it is evident that the practice calls for constant watchfulness.

The most extensively used and by far the most important method of food preservation consists in the use of heat. This method was devised by Appert in 1810. The principles underlying the well-known process of canning, which are, in fact, those of ordinary laboratory sterilization, are: first, the destruction of all germs originally present, and, second, the prevention of subsequent contamination. In practice the process may fail either through incomplete sterilization or through failure to seal hermetically. One of the chief difficulties in practical canning is caused by the presence on vegetables of the spores of anaërobic soil bacteria which are not killed by simple boiling. The sterilizing or "processing" of peas, and especially corn, gives much trouble. If all spores are not killed, gas may be produced and the cans "swell," or the contents may become sour without any external signs of spoiling. In canning corn it has been found necessary to employ a temperature of 250° F. (121° C.) for sixty-five minutes under conditions that insure penetration, in order to preclude spoiling. To this end the cans are placed in a steam retort, in a bath of oil or in water to which certain chemicals, such as calcium salts, are added to raise the boiling-point. In this way the necessary high temperatures are reached. Leaks in the can, as well as insufficient sterilization, may also allow opportunity for bacterial growth. Too thin tin plate, poor soldering material, or imperfect soldering may be responsible for the admission of germs to the sterilized contents. If sterilization is complete at the outset and bacteria do not subsequently find access, canned goods will keep indefinitely without deterioration.

Vinegar-making.—The oxidation of alcohol to acetic acid in weak alcoholic solutions, such as cider and wine, was for a long time thought to be a purely chemical process. It is a fact that when finely divided platinum, the so-called platinum black, is mixed with dilute alcohol, oxidation of the latter occurs, accompanied by the evolution of heat, and at first the "mother of vinegar" was thought to act in a similar manner. The oxidation by platinum black, however, unlike the natural fermentation, takes place much more rapidly at high temperatures, and goes on unimpeded in the presence of an amount of acid that completely prevents the ordinary fermentation process. Pasteur's investigations finally made clear the

share of living micro-organisms in this as in other natural fermentations. The equation

$$\underset{\text{Alcohol}}{C_2H_6O} + \underset{\text{Oxygen}}{O_2} = \underset{\text{Acetic Acid}}{C_2H_4O_2} + \underset{\text{Water}}{H_2O}$$

is only approximately correct, the actual chemical changes in which the micro-organisms participate being much more complicated.

The acetic acid fermentation of cider proceeds most satisfactorily at temperatures of about 18° to 24° C., and is greatly facilitated if vinegar containing "mother of vinegar" is first added to the cider. The "mother," or "Mycoderma aceti," consists of a felt-like scum which commonly forms on the surface of cider or wine during its conversion into vinegar. Different micro-organisms are found in this pellicle, and there is no doubt that several distinct species or varieties are able to bring about the acetic acid fermentation. The acetic acid bacteria usually met with, however, possess certain characteristics in common, such as a marked tendency toward pleomorphism and the production of involution forms. Long filaments which break up into bead-like chains are usually observed, and portions of the filaments are often greatly swollen. The essential physiologic requirement of the acetic acid fermentation is an abundant supply of oxygen, for without this the oxidation of the alcohol cannot be effected. When ordinary casks are used for vinegar-making, they should be not more than two-thirds to three-fourths filled with apple-juice; the outer air should be allowed free access to the cask through a bung-hole, open or stopped with a cotton plug. Special "generators" are in use for hastening the formation of acetic acid by facilitating the absorption of oxygen. A common method in Germany consists in allowing the alcoholic fluid to trickle slowly through a cask filled with shavings impregnated with old vinegar, thus exposing the fluid to the air in thin layers. In the ordinary farm management of vinegar-making freshly pressed apple-juice placed in casks and allowed to stand in the cellar (at a temperature of about 7° to 13° C.) completes the alcoholic fermentation in about five to six months. If the cider is then removed and kept at a warmer temperature, the acetic acid fermentation may be carried out in fifteen to eighteen months, or if left at the lower temperature, in twenty-one to twenty-four months.[1]

[1] Bull. No. 258, N. Y. Agr. Expt. Sta., 1904.

It is sometimes noticed that vinegar allowed to stand for a long time loses its sourness, and that finally all the acetic acid disappears. This is due to the action of certain bacteria (B. xylinum, Browne) which, in the presence of oxygen, split up the acetic acid into other compounds. The deteriorative change can be readily avoided by preventing the free access of oxygen after a sufficient degree of acidity has been reached.

Pure cultures of acetic acid bacteria are not in use in vinegar-making. If undiluted apple-juice with a sufficient sugar content is employed, and if the fermentation processes as above outlined are properly carried out, there seems to be little or no difficulty in obtaining a satisfactory grade of cider vinegar conforming to the legal standard of acetic acid content (4 to 4.5 per cent. acetic acid). In other words, the increased trouble and care incident to working with pure cultures does not at present meet with adequate recompense in an increased value of the commodity.

The Fermentation of Sauerkraut.—Several investigators have reported the presence of organisms in sauerkraut which apparently bear a causal relation to the fermentive process. One of these which seems to be especially significant belongs to the B. coli group. Lactic acid and perhaps other organic acids are produced in abundance by this organism. Gruber[1] has carried out inoculation experiments with pure cultures of the coli-like bacillus isolated by him. The inoculated samples of cabbage are said to have given "a more delicate and aromatic" product than the control samples, and to have reached the desirable stage in a short time. The sauerkraut fermentation and similar souring processes can only be carried on successfully when the air is at least partially excluded from the fermenting mass, since lactic acid formation is inhibited by the presence of oxygen.

The Bakery Fermentations.—In the making of bread, the action of micro-organisms is manifested in several ways, such as (1) The spontaneous fermentation of dough; (2) the use of leaven; (3) the use of yeast; (4) the abnormal fermentations that sometimes cause batches of loaves to spoil.

(1) The natural or spontaneous "rising" which occurs under some conditions in dough made simply with flour and water has been the object of considerable bacteriological study. At first

[1] Gruber: Centralbl. f. Bakt., II., 1909, 22, p. 555.

a specific bacillus, designated as Bacillus levans, was thought responsible for the phenomenon. Later Bacillus levans came to be regarded as merely a variety of the B. coli group, representatives of which are known to be found in some abundance on the surface of grains and in meal. Still further observations again appeared to throw doubt on the nature of the bacteria concerned, for some investigators differentiate Bacillus levans from Bacillus coli on the ground of its slow liquefaction of gelatin, its gas ratio, and other qualities.[1] There seems no reason to suppose that different kinds of aërogenic organisms may not be able under certain conditions to produce the spontaneous rising. More important than the kind of bacteria seem to be (*a*) the carrying out of the process at a low temperature and (*b*) the sugar content of the meal.

(2) The preservation of a small portion of the dough from a previous rising and the thorough kneading of this into fresh dough is a practice of great antiquity. The sour dough, a little of which leaveneth the whole lump, contains, as might be expected, a great variety of micro-organisms. Just as in the case of the micro-organisms in meal, there is some uncertainty about the share of the various organisms in the rising. Yeasts are probably in the main responsible, but at least four different kinds have been found in sour dough, and it has not yet been determined that any one of them is more active than another. Aërogenic bacteria seem to have little or no share in the gas production brought about by inoculation with sour dough. The presence of bacilli of the lactic acid group, however, appears to favor the typical fermentation, since the acid-products not only check the growth of foreign bacteria, but aid in maintaining a suitable reaction for the growth of the yeasts.

(3) The use of top yeast from distilleries, dried or pressed in cakes, is more convenient than the use of sour dough, and, as is well known, is now the general practice. Both yeasts and bacteria occur abundantly in the yeast-cakes, but the former are the active agents in the production of gas, which is practically pure CO_2, not as with the aërogenic bacteria mixtures of CO_2 and H. Theoretically the yeast-cells should be so evenly distributed throughout the fermenting mass that the gas from the alcoholic fermentation is regularly and uniformly generated. This is more easily accomplished with the use of distillery yeast than with sour dough.

[1] Some of the descriptions of B. levans suggest B. cloacæ.

Pure cultures of yeasts have been successfully employed by several experimenters in the preparation of bread. Certain species have been found particularly well adapted for this purpose, for example, the so-called Race XII, discovered in the Berlin Institute for Fermentation Industries. It is said that bread prepared with certain of these pure cultures contains so little butyric and acetic acids that its digestibility is much greater than that of ordinary bread.

(4) The most frequent and most feared abnormal fermentation in the bakery is that which gives rise to the so-called sticky or slimy bread. This is caused by the common "potato bacillus" —Bacillus mesentericus—an organism which forms highly resistant spores and is hence able to survive the temperature reached in the baking process (often not over 100° C.). The spores of this bacillus are sometimes present in large numbers in flour, sometimes also perhaps in the yeast-cakes. The successful combating of the mischief due to the presence of this very resistant organism has been found extremely difficult. An artificial increase of the acidity of the dough is recommended by some experimenters.

The molding of bread is a very common occurrence, and in general the eating of slightly moldy bread does not seem to be accompanied by any serious injury to health. At the same time, the recorded instances of occasional illnesses attributed to this cause are sufficiently numerous to warrant caution in the use of bread in which molds of any kind are growing. Penicillium, Rhizopus, and Aspergillus are the fungi that most commonly attack bread, and there is some evidence indicating that poisonous races of these organisms may exist in some localities. Certain Italian observers claim to have extracted toxic substances from both the spores and the hyphæ of some of these fungi. Pathogenic or toxin-producing races of molds are said to occur in samples of maize suspected of having given rise to pellagra, but no general agreement has been reached among investigators of this malady.

The Retting of Flax and Hemp.—The fibers of certain plants which are used for textile purposes can only in rare cases be separated mechanically from the rest of the plant tissues. Ordinarily such separation is effected by a fermentation process—the so-called retting or rotting. Sometimes the plants are put directly into standing or slowly running water, sometimes they are left

on the ground in such a way that the dew and rain supply the necessary moisture. The process may be accelerated by the use of water warmed to 30°–32° C. The fermentation consists essentially in the dissolving of the substance which binds the fibers together. This cementing substance is largely composed of certain carbohydrates known as pectin bodies, and it is the solution of the pectin which permits the isolation of the bast fibers.

Not all kinds of bacteria are able to accomplish the pectose fermentation. The water-retting of hemp is attributed to the special activity of an anaërobic bacillus, Clostridium amylobacta. The water-retting of flax is also ascribed to a specific anaërobic bacillus ("Granulobacter pectinovorum"). These bacteria hydrolyze the cementing substance by means of an enzyme (pectosinase) which they secrete, and then ferment the simpler products (sugars) which result from the splitting. Some observers have declared that certain common aërobic organisms, such as Bacillus subtilis and B. mesentericus, can bring about the pectose fermentation, but the evidence does not seem to warrant this opinion.[1]

Pure cultures of pectose-fermenting bacteria have been employed by several observers in the retting process, it is said with considerable success. Whether natural or artificial methods be employed, the process should not last too long, otherwise the bast fibers themselves will be injured or destroyed. Such injury may probably be brought about both by the pectose fermenters themselves and by cellulose-fermenting bacteria which may be present.

The Bacterial Destruction of Cellulose.—Under the name cellulose, as is well known, are grouped a variety of nitrogen-free substances which occur especially in the cell-wall of plants and have the general carbohydrate composition indicated by the formula $C_xH_{2y}O_y$. Such substances as cotton, flax, and the Swedish filter-paper used in chemical laboratories are practically pure cellulose. Powerful chemical reagents are not able to effect either the oxidation or the hydrolysis of these bodies, which are completely insoluble. Their disintegration by bacterial agency is, therefore, of special interest. Our knowledge of the cellulose fermentation is due largely to the work of Omelianski.[2]

[1] Tanner, F. W.: Bot. Gaz., 1922, 74, p. 174.

[2] Omelianski: Centralbl. f. Bakt., II, 1902, 8, p. 195; 1904, 11, p. 369; 1904, 12, p. 33.

Two kinds of anaërobic cellulose fermentation have been especially studied: the hydrogen fermentation and the methan fermentation. For the former Omelianski gives the following balance-sheet of an actual experiment:

Cellulose.		Fermentive Products.	
Amount at beginning of experiment	3.4743 gr.	Fatty acids	2.2402 gr.
Amount left at end of experiment	0.1272 gr.	Carbon dioxid	0.9722 gr.
Decomposed during fermentation	3.3471 gr.	Hydrogen	0.0108 gr.
			3.2232 gr.

The hydrogen fermentation of cellulose is brought about by a long, very slender bacillus, with round terminal spores. No growth occurs usually in the ordinary culture-media, though Omelianski has observed on some occasions very minute translucent colonies on potato.

The methan (marsh-gas) fermentation of cellulose yields, like the hydrogen fermentation, a large amount of fatty acids and an even higher proportion of gas (a mixture of carbon dioxid and marsh-gas). About 50 per cent. by weight of the dissolved cellulose is liberated in gaseous form. Morphologically the bacillus of the methan fermentation is very similar to the bacillus of the hydrogen fermentation. Pure cultures have not been obtained. A separation of the two varieties of fermentation, however, is obtained regularly by Omelianski by the method of repeated heatings (75° C. for fifteen minutes), which is based on differences in the life-history of the two organisms. The methan-fermentation organism develops more rapidly than the other variety and gains the upper hand in the early stages of the process. If heat is applied at this stage, the more slowly germinating spores of the hydrogen-fermenting organism are in a resistant stage and survive. On the other hand, successive inoculations of material from the methan fermentation at its height eliminate finally the hydrogen organism, and, when this stage has been reached, heating of a culture no longer yields the results obtained when mixed cultures are so treated. It should be noted that neither of these bacilli stains blue with iodin in any stage of development and that they consequently lack the distinguishing feature of the "Amylobacter" of earlier writers.

In addition to the two common and widely distributed organisms studied by Omelianski, other bacteria, including some aërobic

forms, are able to decompose cellulose, but the conditions of their activity have not been adequately determined. A number of cellulose-dissolving molds are also known.

The destruction of cellulose in the alimentary tracts of some of the higher animals, particularly the herbivora with their enormous length of intestine, is undoubtedly chiefly due to cellulose-dissolving bacteria and not to the digestive fluids. The proportion of cellulose broken up in this way in the alimentary tract may reach as high as 75 per cent. of the amount fed, and since some of the products of this fermentation are utilizable by the animal organism, the nutritional significance of the process is considerable.

The Bacteriology of Silage.—Hunter and Bushnell[1] and Sherman[2] have pointed out the presence of acid-tolerant and acid-producing bacilli in large numbers in silage juice. The character of the fermentations that occur in the curing of corn silage makes it a fair supposition that these organisms are largely concerned in the curing process. They are closely related to the groups of B. bulgaricus (p. 645) and B. acidophilus (p. 646). The acid-producing bacteria of silage are found constantly on corn fodder, so that silage made from corn is always amply seeded with organisms.

[1] Hunter and Bushnell: Science, 1916, 43, p. 318.

[2] Sherman: Jour. Bact., 1916, 1, p. 445.

CHAPTER XXXVII

THE BACTERIA OF AIR, SOIL, AND WATER

Bacteria in Air.—As might be supposed, the number of bacteria in the air bears a close relation to the quantity of larger suspended particles or "dust." There are fewer bacteria in the air of the country than of the city; there are fewer in mountain air than in the air of the lowlands; the air in mid-ocean is nearly germ-free. Pasteur, in an experiment made during the course of his celebrated researches on spontaneous generation, observed that only twelve out of twenty flasks of organic infusion which were opened at a low altitude escaped contamination, while out of twenty opened on the Mer de Glace nineteen escaped. Tyndall's experiment at the Bel Alp in Switzerland was a "yet more emphatic instance of the same kind, 90 per cent. of the flasks opened in the hayloft being smitten, while not one of those opened on the free mountain ledge was attacked." These facts, which indicate the relation of the floating matter of the air to bacterial contamination, have been supplemented by data obtained with the more precise methods of recent investigation.

Several devices for determining the number of bacteria in the atmosphere have been employed. These vary from the simple expedient of exposing agar or gelatin plates to the air for a fixed period, to rather elaborate pieces of apparatus for drawing an accurately measured quantity of air through a filtering substance. The exposure of plates of nutrient media can, of course, give numerical results that are only rudely approximate. Much more definite data have been secured by employing various filtering devices. With the aid of such an apparatus it was found, for example, that in every 10 liters of the outdoor air in the city of Boston in winter there were present about ten to fifteen bacteria capable of growing on the ordinary culture-media and about half as many molds.[1]

The method recommended by the Committee of the American Public Health Association on Standard Methods for Examination

[1] Tucker: Twentieth Ann. Rept. State Board of Health of Mass., 1889.

of Air[1] utilizes a glass tube of 15 mm. in diameter and 70 mm. long with a smaller tube 6 mm. in diameter and 40 mm. long fused into one end (Fig. 181). A plug of cotton on the shoulder where the tubes join supports a layer of sand 10 mm. deep. "The sand should be capable of passing a 100-mesh sieve but not a 200-mesh sieve. The opposite or inlet end of the larger tube is stoppered by a cork stopper (which need not be exactly tight) perforated by a glass tube 6 mm. in diameter and 40 mm. long bent at an angle of 45 degrees to prevent direct precipitation of dust particles into the filter tube.

Fig. 181.—Filter for collecting bacteria from air (Ruehle type). (American Journal of Public Health.)

"Five cubic feet of air should be drawn through the filter by the use of an aspirator of known volume, preferably one of the double or continuous type, or by the use of some form of pump and meter."[2] The sand is shaken out into 10 c.c. of sterile water, and after thorough shaking aliquot portions of the suspension are plated on ordinary nutrient agar.

A simple water aeroscope in which the bacteria are caught by bubbling water is said to give much more satisfactory results than the standard sand method.[3]

Flemming[4] has obtained samples of air high above the earth's surface by the use of balloons, and has found that the air contains living germs up to an altitude of over 4000 meters, although in much smaller numbers at heights above 500 meters than below this altitude. The same observer also found that the bacterial content of the air is much lower in a period of prolonged sunshine than in cloudy weather, that it is especially large at the level of the lower cloud limits, and that chromogenic bacteria and yeasts or torulæ are noticeably abundant.

The kinds of micro-organisms in the air vary somewhat in different localities, but certain forms are pretty uniformly present.

[1] Amer. Jour. of Pub. Health, 1917, 7, p. 54.
[2] Amer. Jour. of Pub. Health, 1917, 7, p. 65.
[3] McConnell and Thomas: Pub. Health Reports, 1925, 40[2], p. 2167.
[4] Flemming: Zeitschr. f. Hyg., 1908, 58, p. 345.

Molds and yeasts are quite common in the atmosphere, and in some situations outnumber the bacteria. Spore-forming bacteria, like the hay bacillus (B. subtilis), are of almost universal distribution, and from their resistance to desiccation are likely to be found in air examinations. Among the saprophytic organisms commonly met with in air are the blue-green mold (Penicillium glaucum), the pigment-producing, yeast-like organism known as "red yeast," and a number of familiar bacteria, including several micrococcus and sarcina forms (Sarcina lutea, a yellow chromogen *et al.*).

Pathogenic micro-organisms, such as the tubercle bacillus and the pyogenic cocci, have been found in the air of hospitals and sick-rooms, but, as a rule, pathogenic bacteria in dry dust are of rare rather than frequent occurrence. Observations and experiments, especially by Flügge and his associates, have shown, however, that bacteria of various kinds may be expelled from the mouth or throat of healthy or sick persons and persist for a time in the immediate surroundings. The fact that minute droplets of moisture or mucus may be discharged into the air by coughing, sneezing, and talking, and may then float about for some time in the neighborhood of the person discharging them, seems to be the most significant result of air examination. Neither typhoid bacilli nor diphtheria bacilli have ever been found in sewer air, which, as a rule, is nearly free from bacteria of all kinds. The absence of germs from the air of sewers is to be explained in large part by the fact that the particles in a current of air passing over a moist surface tend to adhere to the surface and are not easily dislodged. For the same reason the air expired from the lungs contains fewer bacteria than the air inhaled.

Routine bacterial examination of the air of dwellings or schoolrooms has not yielded, and does not seem likely to yield, results of much sanitary importance. The presence of dust particles, to which bacteria chiefly adhere, may be determined in simpler ways.

Bacteria in Soil.—The distribution of bacteria in the soil is naturally dependent upon the presence of organic matter, moisture, and other factors that influence their development and continued vitality. More bacteria are found, for example, in manured soil

than in dry sand. Houston[1] found uncultivated sandy soils to contain on an average 100,000 bacteria per gram, and garden soils 1,500,000, while sewage-contaminated soils might harbor as many as 115,000,000. The upper six inches of the soil are richest in bacteria. Few bacteria are found in undisturbed soil below a depth of 4 to 5 feet. In sand-beds used for filtering sewage a similar vertical distribution is observed; the bacteria are most abundant in the upper layers, and very few are alive in the lower strata.

The supply of bacteria present in the soil is being continually renewed by the excrements of animals, by the bacteria concerned in the various fermentive and putrefactive processes occurring everywhere, and by those that are precipitated out of the air by rain. One is consequently likely to find a large variety of organisms in soil samples, the kinds found at any particular time and place being the result not only of recent additions, but of long-continued selection and adaptation. Unless contamination has been recent and extensive, certain species of bacteria usually predominate in soil. Aërobic spore-forming bacteria (like B. subtilis and allied forms) and also anaërobes (like the bacillus of malignant edema) are particularly characteristic of the normal soil flora. B. proteus (p. 476) is also very common in soil.

Certain spore-forming pathogenic bacteria are found more or less commonly in soil. Spores of the anthrax bacillus may retain their vitality and virulence in the earth for many years, and pasture-lands that are once contaminated with anthrax become practically unsafe for grazing cattle. As already mentioned, the group of pathogenic anaërobes (Cl. tetani, Cl. edematis, *et al.*) finds a congenial habitat in the soil.

Typhoid bacilli sometimes find their way into soil along with human excreta. There is evidence in this case that little or no multiplication takes place, but vitality, on the other hand, may be considerably prolonged, possibly for two or three months.[2] The danger from this source is worth recognizing, since soil contaminated with typhoid bacilli may readily be washed into a water-supply. Con-

[1] Houston: "Report on Chemical and Bacteriological Examination of Soils," London, Local Gov't Bd., 1897–98.

[2] Firth and Horrocks: Brit. Med. Jour., 1902, 2, p. 936; Jordan: Jour. Infect. Dis., 1926, 38, p. 306.

tamination of the water may take place either more or less continuously, or at irregular intervals under certain unusual conditions, as after exceptionally heavy rains. The abundance of B. coli in soil is sometimes, with certain reservations, taken as an indication of the extent and recency of soil pollution. It must be borne in mind, however, that B. coli is commonly present in the intestines of many animals, and that findings must be interpreted with discrimination. Houston[1] has shown that B. coli and other sewage bacteria tend to disappear more or less rapidly from soil to which they are added; that, in a word, a process of bacterial self-purification of soils occurs. Savage[2] also has observed the disappearance of B. coli from "made soil."

The burial of the bodies of persons dying from infectious diseases does not, as has been sometimes surmised, tend to perpetuate pathogenic germs. Rather elaborate experiments by Lösener[3] and others have shown that the longevity of non-spore-bearing organisms under the ordinary conditions of earth burial is not great, a few weeks sufficing for the complete disappearance of S. choleræ, B. diphtheriæ, etc. The hygienic arguments against earth burial, therefore, do not seem to be decisive, whatever be the force of the esthetic and economic objections.

The detailed study of bacteria in soils has been pursued especially in connection with the investigation of agricultural processes. Since the chemical changes produced by bacteria depend both upon the number of organisms present and upon their physiologic activity or "virulence," methods have been devised to determine soil efficiency from these two points of view. Rémy's method[4] consists in adding weighed amounts of soil to nutrient solutions compounded in such a way as to favor the development of various kinds of bacteria, such as the nitrifying, the nitrogen-fixing, the ammonifying, etc. Hiltner and Störmer[5] have attempted to place the determinations on a quantitative basis by the dilution method, using for the inoculation of special solutions constantly decreasing amounts of soil until a point is reached at which the specific physi-

[1] Houston: Rept. Local Gov't Board, London, 1900–01, p. 405; 1901–02, p. 455.

[2] Savage: Jour. Sanit. Inst., 1903, 24, p. 442.

[3] Lösener: Arb. a. d. k. Gesund., 1896, 12, p. 448.

[4] Rémy: Centralbl. f. Bakt., II, 1902, 8, pp. 657, 699, 728, 761.

[5] Hiltner and Störmer: Arb. a. d. k. Gesund., 1903, Biol. Abt., 3, p. 445.

ological action fails to appear. Löhnis,[1] by the use of Hiltner and Störmer's dilution method, found in 1 gram of soil 3,750,000 peptone-decomposing bacteria, 50,000 urea-decomposing bacteria, 50,000 denitrifying bacteria, 7500 nitrifying bacteria, and 25 nitrogen-fixing bacteria. In the same soil only 1,270,000 bacteria developed in soil-extract gelatin plates. Interesting studies have been made upon the effect of adding carbon bisulfid to soils. This substance, which is a powerful germicide, seems to destroy the existing bacterial equilibrium in the soil and to open the way for an entirely new bacterial development. The change thus brought about in the relations of the different groups of soil bacteria favors in some way not wholly understood the accumulation of certain available nitrogen compounds, which are readily utilized by the higher plants.

The Bacteriology of Water.[2]—The bacteria in air are not under suitable conditions for multiplication, but are simply floating about in the forlorn hope, so to speak, that a chance breeze may waft them to a favorable environment; in soil the conditions for development occur only at certain times and places, and in the long run are adapted only for particular species; in water, on the other hand, a proper temperature and abundant food-supply often coexist, and permit the development of a rich and varied bacterial flora. Add to this the fact that many kinds of bacteria are washed into water from air and soil and from the living and dead bodies of plants and animals, and it is seen that almost any germ, pathogenic or saprophytic, might be occasionally or exceptionally found in water. At all events, it is clear that, large as is the number of bacteria that succeed in thriving in water, a far larger number must from time to time make their way into it, to survive there for a longer or shorter period.

The methods used to determine the number and kind of bacteria present in water have been elaborated particularly with a view to the sanitary significance of such an examination. The quantitative examination in its simplest form consists in the enumeration of colonies of bacteria developing upon plates of nutrient gelatin or agar. Specimens of water must be collected in sterilized bottles,

[1] Löhnis: Centralbl. f. Bakt., II, 1905, 14, pp. 2, 3.

[2] The leading book on the bacteriology of water is: Prescott and Winslow "Elements of Water Bacteriology," 4th ed., New York, 1924.

carefully avoiding contamination from the hands or other outside sources. Considerable change may take place in the bacterial content of a water during transportation, even in ice-packed samples,[1] so that the best procedure consists in mixing the water with nutrient agar and plating the mixture within one hour, or preferably one-half hour, after removal of the water from its source. The Committees on Standard Methods for the Examination of Water and Sewage appointed by the Laboratory Section of the American Public Health Association and by the American Water Works Association make the following recommendations:[2]

The final reaction for broth, gelatin and agar shall be between P_H 6.2 and P_H 7.0 . . . Samples for bacterial analysis shall be collected in bottles which have been cleansed with great care, rinsed in clean water and sterilized at a temperature not less than 160° C. for not less than one hour or in a steam autoclave at 15 lbs. pressure for one-half hour . . . All sample and dilution bottles shall be shaken vigorously 25 times before samples are removed for plating. Plating shall be done immediately after the dilutions are made. One c.c. of the sample or dilution shall be used for plating and shall be placed in the Petri dish first. Ten c.c. of liquefied medium at a temperature of 40° C. shall be added to the 1 c.c. of water in the Petri dish. The cover of the Petri dish shall be lifted just enough for the introduction of the pipette or culture medium, and the lips of all test-tubes or flasks used for pouring the medium shall be flamed. The medium and sample in the Petri dish shall be thoroughly mixed and uniformly spread over the bottom of the Petri dish by tilting and rotating the dish. All plates shall be solidified as rapidly as possible after pouring and placed immediately in the appropriate incubator . . . Gelatin plates shall be incubated for 48 hours at 20° C. in a dark, well-ventilated incubator in an atmosphere practically saturated with moisture. Agar plates may be used for counts made either at 20° C. or 37° C. The time for incubation at 20° shall be 48 hours and at 37°, 24 hours. The incubator shall be dark, well ventilated and the atmosphere shall be practically saturated with moisture. Glass covered plates shall be inverted in the incubator . . . In preparing plates, such amounts of the water under examination shall be plated as will give from 30 to 300 colonies on a plate; and the aim should be to always have at least two plates giving colonies between these limits. Where it is possible to obtain plates showing colonies within these limits, only such plates should be considered in recording results, except where the same amount of water has been planted in two or more plates, of which one gives colonies within these limits, while the others give less than 30 or more than 300. In such case, the result recorded should be the average of all the plates planted with this amount of water. Ordinarily it is not desirable to plant more than 1 c.c. of water in a plate; therefore, when the total number of colonies developing from 1 c.c. is less than 30, it is obviously necessary to record the result as observed, dis-

[1] Jordan and Irons: Reports and Papers of Amer. Pub. Health Assoc., 1899, 25, p. 564.

[2] Standard Methods of Water Examination, 6th edition, 1925.

regarding the general rule given above. Counting shall in all cases be done with a lens of $2\frac{1}{2}$ diameters' magnification, $3\frac{1}{2}$ X, with a focal distance of $3\frac{1}{2}$ inches. The Engraver's Lens No. 146 made by the Bausch & Lomb Optical Company fills the requirements and is a convenient lens for the purpose . . . In order to avoid fictitious accuracy and yet to express the numerical results by a method consistent with the precision of the work, the numbers of colonies of bacteria per c.c. shall be recorded as follows:[1]

NUMBER OF BACTERIA PER C.C.

From	1	to	50	shall be recorded	as found
"	51	"	100	to the nearest	5
"	101	"	250		10
"	251	"	500		25
"	501	"	1,000		50
"	1,001	"	10,000		100
"	10,001	"	50,000		500
"	50,001	"	100,000		1,000
"	100,001	"	500,000		10,000
"	500,001	"	1,000,000		50,000
"	1,000,001	"	10,000,000		100,000

This applies to the gelatin count at 20° C. and to the agar counts at 20° C. and 37° C.

The results obtained by the use of quantitative methods are in all cases relative and approximate rather than absolute and exact. Certain bacteria, such as the strict anaërobes, do not grow under the conditions in which the plates are incubated, and others, like the nitrifying organisms, have peculiar nutritional requirements and do not develop on the ordinary media.

Nevertheless the "colony counts" or "numbers of bacteria" reported by different observers are in some degree comparable, especially when standard methods are employed.

As might be supposed on *a priori* grounds, very large numbers of bacteria are found in sewage and sewage-polluted waters, whereas very few occur in the water of most springs or deep wells. River-water contains, as a rule, more bacteria than lake- or pond-water, the difference being due in part at least to the sedimentation that occurs in quiet waters. The following table includes some representative determinations of the number of bacteria in water:

[1] Standard Methods for the Examination of Water and Sewage, 1925, 6th edition.

Source.	Number of Colonies per Cubic Centimeter.	Authority.
Thames River	277 (Apr.)–2075 (Jan.)	Houston.
Illinois River at Ottawa (about 55 miles below mouth of Chicago Drainage Canal)	6300–8200 (May)	Jordan.
Potomac River	750 (May)–11,500 (Mar.)	Longley.
Mississippi River at New Orleans	805 (Aug.)–3597 (Apr.)	Weston.
Loch Katrine	74	Frankland.
Lake of Lucerne	8–51	Frankland.
Lake Michigan near Chicago	68–2000	Jordan.
Deep well-waters	0–12	Prescott and Winslow.
Spring-water (av. of 54 samples	41	Mass. State Bd. of Health.
Sewage (Boston)	712,000 (Dec.)–11,487,500 (Sept.)	Winslow.

The degree of sanitary significance attaching to such data has been the subject of some difference of opinion and some confusion. The belief is widespread among the general public that the sanitary character of a water can be estimated pretty directly by the number of bacteria it contains. Taken by itself, however, it must be admitted that the number of colonies which develop when a given sample of water is plated affords no secure basis for judging potability. A pure spring-water containing at the outset less than 100 bacteria per cubic centimeter may come to contain tens of thousands per cubic centimeter within twenty-four to forty-eight hours, after standing in a clean glass flask at a fairly low temperature. There is no reason for supposing that the wholesomeness of the water has been impaired in any degree by this multiplication of bacteria.

As a matter of fact, like the sanitary chemical analysis of water, the quantitative bacterial analysis has only an empirical value. Experience in a broad way has shown that most natural waters known to be pure contain relatively few bacteria capable of developing by the usual methods, whereas sewage and sewage-polluted waters contain large numbers. Some observers of wide experience are inclined to hold that natural waters, which are found by approved methods to contain more than 1000 bacteria per cubic centimeter,

should be regarded as distinctly suspicious. A turbid river-water, however, may be relatively unpolluted, and yet at times contain several thousand bacteria per cubic centimeter.

It has been shown in the chapters on typhoid fever and cholera that the epidemiologic evidence connecting the use of sewage-polluted water with the causation of specific disease is of a very definite and cogent character. At the time when methods for the bacterial examination of water first came into use it was confidently expected that with their aid it would be possible to discover the presence of specific pathogenic bacteria, and so obtain more or less precise information as to the wholesomeness of a water. These expectations have never been realized, partly because of the great difficulty, in spite of the invention of many ingenious methods, in picking out specific micro-organisms from among immense numbers of sewage bacteria, partly because the life of the typhoid bacillus and the cholera spirillum in water is short, and examination of a water known to have dealt disease or suspected of having done so is often so long delayed that any disease germs that may have been originally present have perished. For these reasons the search for specific pathogenic bacteria in water is rarely crowned with success, and their real or apparent absence affords no good ground for judging the general safeness of the water examined. As pointed out above, the actual number of bacteria in a water is also no absolute criterion of wholesomeness, although, taken together with other factors concerning the source and history of the water, it may prove of service in forming an opinion.

The practical failure of colony enumeration and of the search for specific disease germs to disclose important sanitary relations has led to other attempts to correlate the bacterial content of a water with its sanitary quality. The most widely used and, by general consensus, the most valuable of these tests is the "B. coli test." This is based upon the circumstance that the colon bacillus, B. coli, is a common inhabitant of the human intestine, and is found in great abundance in sewage.[1] Its close biological relationship to the typhoid bacillus and the fact that like the latter organism it finds its way into sewage chiefly from the discharges of the human body render its presence, especially when in large numbers, peculiarly suggestive.

[1] As many as 100,000 per cubic centimeter in fresh sewage.

The test for the presence of members of the coli-aerogenes group is thus dealt with in the 6th Report of the Committee on Standard Methods (1925);

1. Introduction and Definitions

It is recommended that the coli-aërogenes group be considered as including all gram-negative non-spore-forming bacilli which ferment lactose with gas formation and grow aërobically on standard solid media.

The formation of 10 per cent. or more of gas in a standard lactose broth fermentation tube within 24 hours at 37° C. is presumptive evidence of the presence of members of this group, since the majority of the bacteria which give such a reaction belong to the group.

The appearance of aërobic lactose-splitting colonies on Endo or eosin methylene blue plates made from a lactose broth fermentation tube in which gas has formed, confirms to a considerable extent the presumption that gas formation in the fermentation tube was due to the presence of members of the coli-aërogenes group.

To complete the demonstration of the presence of organisms of this group, it is necessary to show that one or more of these aërobic plate colonies consist of non-spore-forming bacilli which, when inoculated into a lactose broth fermentation tube, form gas.

It is recommended that the standard tests for the coli-aërogenes group be either the Presumptive, the Partially Confirmed, or the Completed test as hereafter defined, each test being applicable under the circumstances specified.

2. Presumptive Test

(*a*) *Inoculation.*—Inoculate a series of lactose broth fermentation tubes with appropriate graduated quantities of the water to be tested. In every fermentation tube there must always be at least twice as much medium as the amount of water to be tested. When required to examine larger amounts than 10 c.c. as many tubes as necessary shall be inoculated with 10 c.c. each.

(*b*) *Incubation and Reading.*—Incubate these tubes at 37° C. for 48 hours. Examine each tube at 24 and 48 hours, and record gas formation. The records should be such as to distinguish between:

(1) Absence of gas formation.

(2) Formation of gas occupying less than 10 per cent. of the inverted vial.

(3) Formation of gas occupying more than 10 per cent. of the inverted vial.

More detailed records of the amount of gas formed, though desirable for the purpose of study, are not necessary for carrying out the standard tests prescribed.

(*c*) *Positive Presumptive Test.*—Formation within 24 hours of gas occupying more than 10 per cent., the incubation shall be continued to 48 hours. The presence of gas in any amount in such a tube at 48 hours constitutes *a doubtful test.* (An arbitrary limit of 48 hours' observation doubtless excludes from consideration occasional members of the coli-aërogenes group which form gas very slowly, but for the purpose of a standard test the exclusion of these occasional slow gas-forming organisms is considered immaterial.)

3. Partially Confirmed Test

(*a*) *Preparation of Plates.*—Make one or more Endo or eosin methylene blue plates from the tube which shows gas formation from the smallest amount of water tested. It is desirable to make transfers to Endo or eosin methylene blue as soon as possible after gas formation occurs. If gas formation occurs at the end of 24 hours make transfers at that time. If at the end of 48 hours gas has formed in tubes containing less of the sample of water than at 24 hours, transfers should be made from these tubes. (For example, if the water has been tested in amounts of 10 c.c., and 0.1 c.c., and gas is formed in 10 c.c. and 1 c.c., not in 0.1 c.c., the test need be confirmed only in the 1 c.c. amount.)

(*b*) *Incubation of Plates.*—Incubate the plates at 37° C. for 18 to 24 hours.

(*c*) *Results, Typical and Atypical.*—(1) If typical colonies have developed upon the plate within this period, the confirmed test may be considered positive. (2) If, however, no typical colonies have developed within 24 hours, the test cannot yet be considered definitely negative, since it not infrequently happens that members of the coli-aërogenes group fail to form typical colonies on Endo or eosin methylene blue plates, or that the colonies develop slowly. In such case, it is always necessary to complete the test as directed under 4, (*b*) and (*c*).

4. Completed Test

(*a*) *From Typical Plates.*—From the Endo or Eosin methylene blue plates made as prescribed under 3, fish at least two typical colonies, transferring each to an agar slant and a lactose broth fermentation tube.

(*b*) *From Atypical Plates.*—If no typical colonies appear upon the plate within 24 hours, the plate should be incubated another 24 hours, after which at least two of the colonies considered most likely to be organisms of the coli-aërogenes group, whether typical or not, shall be transferred to agar slants and lactose broth fermentation tubes.

(*c*) *Interpretation of* (*a*) *and* (*b*).—The lactose broth fermentation thus inoculated shall be incubated until gas formation is noted,—the incubation not to exceed 48 hours. The agar slants shall be incubated at 37° C. for 24 hours, when a microscopic examination shall be made of at least one culture, selecting the one which corresponds to one of the lactose broth fermentation tubes which has shown gas formation.

The formation of gas in lactose broth and the demonstration of gram-negative non-spore-forming bacilli in the agar culture shall be considered a satisfactory completed test, demonstrating the presence of a member of the coli-aërogenes group.

The absence of gas formation in lactose broth or failure to demonstrate gram-negative non-spore-forming bacilli in a gas-forming culture constitutes a negative test.

APPLICATION AND OUTLINE OF PRESUMPTIVE, PARTIALLY CONFIRMED, AND COMPLETED TESTS

1. The Presumptive Test

(*a*) When definitely positive, that is, showing more than 10 per cent. of gas in 24 hours, is sufficient:

(1) As applied to all except the smallest gas-forming portion of each sample in all examinations.

(2) As applied to the smallest gas-forming portion in the examination of sewage or of water showing relatively high pollution, such that its fitness for use as drinking water does not come into consideration. This applies to the routine examination of raw water in connection with control of the operation of purification plants.

(*b*) When definitely negative, that is, showing no gas in 48 hours, is final and therefore sufficient in all cases.

(*c*) When doubtful, that is, showing gas less than 10 per cent. (or none) in 24 hours, with gas either more or less than 10 per cent. in 48 hours, must always be confirmed.

2. The Partially Confirmed Test

(*a*) When definitely positive, that is, showing typical plate colonies within 24 hours, is sufficient:

(1) When applied to confirm a doubtful presumptive test in cases where the latter, if definitely positive, would have been sufficient.

(2) In the routine examination of water supplies where a sufficient number of prior examinations have established a satisfactory index of the accuracy and significance of this test in terms of the completed test.

(*b*) When doubtful, that is, showing colonies of doubtful or negative appearance in 24 hours, must always be completed.

3. The Completed Test

The completed test is required as applied to the smallest gas-forming portion of each sample in all cases other than those noted as exceptions under the "presumptive" and the "partially confirmed" test.

The completed test is required in *all* cases where the result of the partially confirmed test has been doubtful.

Using the term "B. coli" broadly, the interpretation of the results of the test is a matter on which there is now general agreement. It must be remembered that the relative abundance, rather than the presence, of colon bacilli, is the essential feature of this test. The discovery of a single B. coli in 50 c.c. of a water or even occasionally in 5 c.c. affords no reasonable ground for casting suspicion upon the character of the water. The possibility of sporadic contamination with colon bacilli derived, not from man, but from domestic animals or birds, must always be kept in mind. Manured fields and pastures filled with grazing cattle or sheep are likely sources of colon bacilli, and may give rise to mistaken inferences if the environmental examination of a water-supply is neglected. Considerable numbers of colon bacilli in a water, however, are always suggestive of marked fecal contamination by man or animals.

The B. coli content of shellfish has been used as an index of sanitary quality. The undiluted shell liquor is subjected to a series of tests similar to those used in water examination.[1] Different interpretations may be placed upon the bacterial score (B. coli number) in shell oysters and shucked stock. Sanitary inspection and control of the oyster beds is a prime consideration.

General reliance upon the principle of the B. coli test has led in recent years to its wide-spread application. It is not only almost invariably employed in the routine examinations of water from suspected sources, but has also been resorted to for the study of special problems, such as the efficiency of sand filters[2] and the self-purification of streams,[3] as well as the pollution of oyster-beds.[4] On the whole, it is felt by water analysts that greater weight can be attached to the results of the B. coli test in the hands of an experienced observer than to any other laboratory determination, bacterial or chemical.

An advisory committee appointed by the U. S. Treasury Department to recommend standards of purity for drinking-water supplied to the public by common carriers engaged in interstate traffic, has formulated the following standard:[5]

"(1) Of all the standard (10 c.c.) *portions* examined in accordance with the procedure specified below, not more than 10 per cent. shall show the presence of organisms of the *B. coli* group.

"(2) Occasionally three or more of the five equal (10 c.c.) *portions* constituting a single standard *sample* may show the presence of *B. coli*. This shall not be allowable if it occurs in more than:

"(*a*) Five per cent. of the standard samples when twenty (20) or more samples have been examined.

"(*b*) One standard sample when less than twenty (20) samples have been examined.

"Note.—It is understood that in the examination of any water supply the series must conform to *both the above requirements*, (1) and (2). For example, where the total number of samples is less

[1] Standard Methods for the Examination of Shellfish: Am. Jour. Pub. Health, 1922, 12, p. 574.

[2] Thirtieth and Thirty-first Rept. Mass. State Bd. of Health, 1898, 1899

[3] Jordan: Jour. Exper. Med., 1900, 5, p. 271.

[4] Klein, E.: Report of Experiments and Observations on the Vitality of the Bacillus of Typhoid Fever and of Sewage Microbes in Oysters and Other Shell-fish, London, 1905.

[5] Public Health Reports, 1925, 40, p. 693.

than six, the occurrence of positive tests in three or more of the five portions of any single sample, although it would be permitted under requirement (2), would constitute a failure to meet requirement (1).

"*Definition.*

"*The B. coli group* is defined, for the purposes of this test, as in Standard Methods of Water Analysis, American Public Health Association, New York, 1923, and the procedures for demonstration of organisms of this group shall conform to those of the "completed test" as therein specified.

"The *standard portion* of water for this test shall be ten cubic centimeters (10 c.c.).

"The *standard sample* for this test shall consist of five (5) standard *portions* of ten cubic centimeters (10 c.c.) each . . .

"The bacteriological examinations which have come to be generally recognized as of most value in the sanitary examination of water supplies, are:

"(1) The count of total colonies developing from measured portions planted on gelatin plates and incubated for 48 hours at 20° C.

"(2) A similar count of total colonies developing on agar plates incubated for 24 hours at 37° C. (or in some laboratories incubated 48 hours of 20° C.).

"(3) The quantitative estimation of organisms of the *B. coli* group by applying specific tests to multiple portions of measured volume.

"Of these three determinations, the test for organisms of the *B. coli* group is almost universally conceded to be the most significant, because it affords the most nearly specific test for the presence of fecal contamination. The committee has, therefore, agreed, after full consideration, to include only this test in the bacteriological standard recommended, believing that neither the 37° C. nor the 20° C. plate count would add information of sufficient importance to warrant complicating the standard by including them in the required examination. The omission of plate counts from the standard is not to be construed, however, as denying or minimizing their importance in routine examinations made in connection with the control of purification processes. On the contrary, the committee wishes to record its opinion that one or both plate counts

are of definite value in such examinations, and to emphasize that it is chiefly in the interest of simplicity that they have been omitted from the standard here proposed."

Some attempts have been made to discover other organisms besides B. coli that are equally indicative or more indicative of sewage contamination. Streptococci, for example, have been supposed by some observers to show that recent and hence specially objectionable pollution has occurred, but the weight of available evidence is against this view. The longevity of streptococci in water is sometimes greater, sometimes less, than that of colon bacilli. The lack of suitable differential characters in the group of streptococci renders the use of these organisms as an index of sewage pollution a procedure of doubtful value.

Among other bacteria found frequently in sewage and polluted water may be mentioned the Proteus group (p. 702); B. cloacæ, B. welchii (p. 403), and B. subtilis (p. 276). These forms occur also in pure water, but much less commonly. A host of different microbes have been described as inhabiting uncontaminated water; one of the most common and abundant of these is B. fluorescens liquefaciens, an organism resembling B. pyocyaneus in nearly all respects except that the former does not produce a blue pigment.

The great majority of bacteria in water are killed by freezing, hence ice always contains but a fraction of the number in the water from which it was formed. Over 90 per cent. both of the ordinary water bacteria and of typhoid bacilli die within a few hours, and a progressive decline in numbers then take place, less than 1 per cent. of typhoid bacilli surviving at the end of a week of freezing, according to experiments. Ice stored for six months is practically sterile. Outbreaks of typhoid fever have rarely been traced to the use of ice, although in at least one case the evidence of ice transmission seems quite conclusive.[1] Danger of typhoid infection from the use of ice in drinking-water is always less than from the use of water from the same source.

When by bacterial examination or otherwise a water is known to be unsafe for consumption, the question arises as to ways and means of artificial purification. There are a number of useful methods of purifying water, differing according to the amount and character of the water to be treated. Large public water-supplies in Europe are commonly purified by sand-filtration. The sand

[1] Hutchings and Wheeler: Amer. Jour. Med. Sci., 1903, 126, p. 680.

filters are constructed so that the water passes through from 1 to 5 feet of sand supported upon carefully graded layers of gravel (Fig. 182). The rate of filtration must be accurately regulated, and the efficiency of operation controlled by frequent bacterial tests of the effluent.[1] Such filters are now in operation in Lawrence, Mass., Albany, N. Y., Washington, D. C., Philadelphia, Pa., and other cities in the United States. At Lawrence water pumped from the Merrimac River is applied to the filters; the river-water contained in 1905 an average of 12,700 bacteria per cubic centimeter, and the effluent from the filters only 70, while the percentage of 1 c.c. samples containing B. coli was diminished from 100 per cent. in the raw water to 4.7 per cent. in the filtered water. The real sanitary efficiency of the filtering process is further attested by the

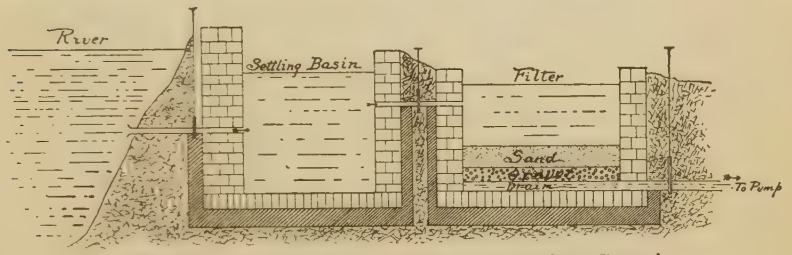


Fig. 182.—Cross-section of filter plant (after Hazen).

typhoid fever death-rate in Lawrence, which sank from an average of 9.24 in the fourteen years before the filter was installed to an average of 3.02 in the years 1894 to 1904.

Highly turbid waters, such as those of the Mississippi, Ohio, Missouri, and other western rivers, require to be clarified as well as purified. So-called mechanical or rapid filters are often used where a simple sand filter could not yield a clear effluent, or would become quickly clogged. The mechanical filters, of which there are many, require for their operation the use of a coagulant. Either sulfate of aluminum or ferric sulfate is commonly employed for this purpose. On the addition of the coagulant to the water, a flocculent precipitate is formed which carries down with it a large part of the suspended matter. When properly managed, a high degree of bacterial efficiency may be reached with mechanical filters. They have the advantage of being able to treat a large quantity of water upon a relatively small filtering area, and also of removing minute

[1] Thirtieth and Thirty-first Rept. Mass. State Bd. of Health, 1898, 1899.

particles of clay which the ordinary sand filter allows to pass. A combination of the use of a coagulant with the hygienically efficient sand filters has been recommended for use in some localities where high turbidity prevails only during certain periods.

Methods of purifying water by chemical treatment are steadily increasing in use. Ozone was one of the first germicides to be used in this way, and very effective generators have been devised for producing ozone on a large scale and introducing it into the water to be treated. The germicidal power of the gas is high and, where a clear, slightly polluted water is to be treated, the results are excellent. Of late the ozone treatment has lost ground in competition with the method of chlorination, which is equally efficacious and very much cheaper.[1]

Bleaching powder, the active agent of which is hypochlorite of calcium, was extensively used for a time, (*ca.* 1908–1914), in water purification in this country, but has been superseded by liquid chlorine. Chlorine is usually applied to public water supplies in accurately measured dosage, the amount used being determined by the character of the water treated. It is now believed that the germicidal effect is due to the direct action of chlorine and its derivatives and not to nascent oxygen as was at one time supposed. The tastes and odors in chlorinated waters may be due to an overdose of chlorine caused by inadequate control methods or to the action of chlorine upon certain organic compounds found in the water treated. The most objectionable odors appear to be those caused by the action of chlorine on certain manufacturing wastes.[2]

In practice this mode of water treatment has obtained wide extension. In 1924 over 3,750,000,000 gallons of water per day were being chlorinated in more than 3000 cities and towns in the United States. Probably about one-third of the population of the United States is today efficiently protected against water-borne disease by chlorination. When liquid chlorine is added to ordinary surface water, clear and not highly contaminated, in the proportion of about 0.5 to 1 part of "available chlorine" per million gallons, the ordinary intestinal bacteria are destroyed, including such pathogenic forms as the typhoid bacillus. In cities like Minneapolis and Milwaukee, where the chlorine treatment has been applied to the city

[1] Water Works Practice, 1925, p. 265.
[2] Water Works Practice, 1925, pp. 173–188.

water-supply, a distinct reduction of typhoid fever has occurred. In Minneapolis only 20 deaths from typhoid fever were reported in the first ten months of 1911, as compared with 159 in the corresponding period of 1910. Portable hypochlorite plants have been devised,[1] and may be quickly shipped to places where a sudden emergency arises.

Ultraviolet rays have also been used in some French cities for sterilizing water-supplies, but the method has not come into general use.[2] In the United States at present the ultraviolet ray process is chiefly used in the treatment of swimming pools.

When, as is too often the case, a public supply is known to be impure, domestic or house filters are often resorted to. Many of the articles sold as filters or water purifiers have little sanitary value, and at most remove excessive color or turbidity from the water without materially increasing its safety. Certain kinds of small filters are attached directly to the tap, and the water is filtered under pressure; others are simple gravity filters on which the water is poured and through which it percolates slowly. The principle underlying the operation of all the best house filters operated under pressure is essentially the same; namely, straining out of the bacteria. Since bacteria can pass freely through a thin layer of loose or coarse material, only those filters made of exceedingly compact material can achieve the end desired. Among the best-known filters of this class are the Pasteur-Chamberland (baked clay) and the Berkefeld (infusorial earth). Both of these have been repeatedly demonstrated to be efficient when operated under the conditions obtaining in a bacteriologic laboratory, and yield sterile effluents, the Berkefeld for several days, the Pasteur-Chamberland for somewhat longer. Under actual working conditions in the household and elsewhere, the use of the compact filters not infrequently presents certain difficulties. The yield of these filters, particularly and necessarily of the more efficient types, is always scanty, and if the details of connection and cleansing are intrusted to unskilled or negligent hands, the filter may become entirely useless. A good deal of time and care are needed to insure that a battery of these tubes in a large school or factory is kept continually in good working order. Daily inspection by competent and trustworthy persons

[1] Childs and Whittaker: Eng. News, 1911, 65, p. 402.

[2] See Eng. News, 1911, 66, p. 525; Water Works Practice, 1925, p. 264.

is indispensable, and bacterial tests should be made at frequent intervals.

"It might be thought that nowadays most people understood the proper use of filters on the Pasteur principle; it has, however, been found that in regimental soda-water factories in India (managed and supervised by regimental officers), where the water was known to be dangerous and in need of efficient filtration, the filter *bougies* have been fixed in such a way as to permit of the water passing through the joint, instead of through the wall, of the bougie; this being done in order to get a more rapid flow . . .

"During fifteen years a large number of filters have been used in barracks and hospitals; they have proved to be wonderfully serviceable. But they require minute attention; and every fortnight taking to pieces, cleaning, inspection, replacement of perhaps 150 fragile tubes and receptacles. In default of such attention, the filters rapidly become useless or even dangerous."[1]

Gravity or low-pressure house filters with a 12-inch sand layer may be constructed on the same principle as the large sand filters above described; when properly operated, they constitute a considerable safeguard. Animal charcoal, by removing color, gives brilliancy to the water, but is not in other respects suitable for a filtering substance.

At all times the safest method of purifying water is by boiling. It is not necessary to render the water sterile, that is, to free it altogether from the spores of the hay bacillus or other harmless organisms. Boiling for five minutes is quite sufficient to destroy with certainty the typhoid bacillus and allied forms, as well as the cholera spirillum. Anthrax infection by way of drinking-water, although theoretically possible, is so rare as to be practically unknown; even anthrax spores, however, are killed by ten minutes' boiling. The flat taste of freshly boiled water may be removed by pouring the water a few times back and forth from one vessel to another, or by passing it through an inexpensive sand or sandstone gravity filter, which also removes the larger suspended particles. When water-borne disease is prevalent, or when a water-supply is notoriously impure or exposed to chance of infection, boiling is the only wholly safe procedure.

[1] Jour. Roy. Army Med. Corps, 1904, 2, pp. 706, 751.

CHAPTER XXXVIII

THE BACTERIAL DISEASES OF PLANTS

Although the diseases of plants demonstrated to be caused by bacteria are perhaps not so numerous as those of animals, there can be no doubt that bacteria play a much more important part in plant pathology than was at one time supposed. It seems likely that there are many bacterial infections still unrecognized. Up to the present over forty have been described. Only a few of the better-known plant diseases can be considered here.

Pear Blight (Bacillus amylovorus).—In 1880 Burrill[1] found a motile bacillus constantly present in the freshly blighted twigs of pear trees. No trace of fungous growth was present in the infected region, and the bacillus was always found pushing into the sound tissues in advance of visible browning and death. Inoculations made with material taken directly from diseased tissues produced the blight in healthy fruit trees. Later experiments made by Arthur and by Waite,[2] using more modern methods, confirmed the essential features of Burrill's work. Pure cultures of B. amylovorus inoculated by means of delicate needle punctures into young shoots almost always produce the disease in the inoculated trees, while uninoculated trees in the neighborhood remain healthy. Careless pruning may sometimes be responsible for the orchard transfer of the disease. Waite has isolated B. amylovorus from the mouth parts of bees that had visited blighted pear flowers, has seen bees pass from such flowers to healthy ones, and blight subsequently appear in the latter. The extent to which insects actually spread the disease is, however, doubtful. Stevens and his associates[3] have advanced strong evidence to show that wind is the main agent of transmission.

Apple, quince, and apricot trees are also susceptible to this infection. Some varieties of pears show higher resistance than others. Wild native trees, such as crabs, hawthorns, and plums,

[1] Burrill: Proc. Am. Assoc. Adv. Sci., 1880, 29, p. 583.

[2] See Smith, E. F.: Centralbl. f. Bakt., II, 1899, 5, p. 810.

[3] F. L. Stevens, W. A. Ruth, C. S. Spooner: Science, Nov. 1, 1918, p. 449.

are liable to infection and may constitute an important source of orchard trouble.

B. amylovorus possesses, according to Waite,[1] the following characteristics: It is a motile, non-capsulated bacillus, about 0.6 to 0.8 μ in diameter, and from 1 to 6 μ in length. The flagella are disposed peritrichally. In broth a marked turbidity is produced and a delicate pellicle is formed which finally breaks up and sinks. Gelatin is liquefied, although not with great rapidity. Upon agar, potato, and other solid media the color of the growth is milky white. Acid is produced in various carbohydrate solutions, more vigorously with maltose than with saccharose, dextrose, or levulose. No gas

Fig. 183.—Muskmelon plant inoculated with a pure culture of B. tracheiphilus (Erwin F. Smith).

is formed in the fermentation tube. Starch is not fermented. No pigment is formed and the cultures emit no odor.

The Wilt Disease of Cucurbita (Bacillus tracheiphilus[2]).—Cucumbers, muskmelons, pumpkins, and squashes are liable to a disease characterized by the wilting of the vines, followed by shriveling and death (Fig. 183). It is caused by a bacillus whose growth fills up the water-ducts or tracheæ with a white, viscid mass. If a cucumber leaf is lightly pricked with a needle dipped in a pure culture of B. tracheiphilus, the bacteria make their way down the

[1] Waite: Science, N. S., 1898, 8, p. 692.

[2] Smith, Erwin F.: Centralbl. f. Bakt., II, 1895, 1, p. 364.

petiole of the leaf into the stem, where enormous multiplication occurs in the water-ducts. The first signs of the disease occur usually after about a week and always first on the inoculated leaf. In nature the disease appears to be spread mainly, if not solely, through the wounds inflicted by insects, such as the striped cucumber beetle and the common squash bug. Erwin Smith produced the disease by allowing these insects to feed on diseased vines and subsequently on healthy ones; this observer never saw the disease escape to control plants during greenhouse experiments, except through the exclusive agency of insects as disseminators.

B. tracheiphilus grows in culture-media, but is a sensitive species. Upon agar a white, extremely viscid growth is produced. In milk no visible change occurs. The growth on potato resembles that of the typhoid bacillus. Acid is formed from glucose and saccharose. The optimum temperature for development is between 20° and 30° C.; no growth takes place at 37° C. Gelatin is not liquefied.

Brown Rot of Tomato, Egg Plant, and Potato (Bacillus solanacearum[1]).—A disease which affects a number of solanaceous plants[2] and is somewhat similar to the cucumber wilt in its general symptoms, *i. e.*, wilting, is due to a different micro-organism. After a premonitory wilting of one or more shoots, the stem, especially in young plants, shrivels and finally changes to brown or black. The vessels of the affected vascular bundles are filled with myriads of bacilli. In the potato plant the bacilli spread by way of the vascular bundles to the tubers, which are attacked and destroyed. Smith and others believe that a large part of the common potato rot of the northern United States is due to this bacterial infection. In this disease, as in the cucumber wilt and pear blight, the part played by insects in the rôle of bacillus-carriers seems to be of the first importance. Experiments made with potato beetles show that when the insects are fed on diseased plants, they become capable of transmitting the rot to healthy ones. It seems possible that other leaf-eating insects may be the means of transmitting the disease. Injury to roots during transplanting is also a source of infection.

Bacillus solanacearum grows on the ordinary culture-media with formation of a dirty white pigment which discolors the substratum.

[1] Smith, Erwin F.: Bull. No. 12, Div. of Veg. Physiol. and Path., U. S. Dept. of Agric., 1896.

[2] The tobacco plant and the nasturtium are also liable to this infection.

The growth is not viscid, or only slightly so. Gelatin is not liquefied. Acid is not formed from any of the sugars. The fat of milk is dissolved and the medium becomes strongly alkaline and translucent. The bacillus grows well at 37° C. The micro-organism is not pathogenic for the cucurbitaceous plants, and, conversely, attempts to infect the potato and tomato with the bacillus of cucumber wilt (B. tracheiphilus) have given negative results.

A serious disease of the tobacco plant in Florida and North Carolina (Granville wilt of Stevens and Sackett) is caused by the same organism (Smith). In this case infection probably first occurs in the root. Observations in Sumatra indicate that land infected with nematodes which attack the root or base of the plant stem is particularly subject to the disease, so that the bacteria are probably brought into contact with the plant tissues through the agency of these parasitic worms. Tomato plants can be successfully inoculated with the micro-organism of tobacco wilt and vice versâ. Plants of one species grown in soil previously occupied by affected plants of another species of the same family also contract the disease.

The Basal Stem-rot of Potato (Bacillus phytophthorus[1]).— We are indebted to Otto Appel, of Berlin, for our first exact knowledge of this disease. In the United States Appel's work has been repeated and confirmed by Smith.[2] The stems of the potato rot off at the surface of the earth; the tubers are rotted in the earth and also subsequently in storage. Often tubers which appear to be sound or nearly sound externally are badly rotted internally. This disease is widely prevalent in the United States and in Europe. It is much less a vascular disease than the preceding.

The organism causing it is a white, peritrichial bacillus, having the following characteristics (Smith): Clouds bouillon very rapidly at 30° C.; on thin sown gelatin plates, large circular colonies; gelatin is slowly liquefied; negative gram stain; does not produce indol; no gas from potato juice, or Witte's peptone in water with grape-sugar or cane-sugar; reddens litmus milk, and throws down a pleasant-smelling curd after five or six days; does not grow in

[1] Appel, Otto: "Untersuchungen ü. d. Schwarzbeinigkeit, etc.," Arb. Biol. Abt. d. k. Gesundh., Berlin, 1903.

[2] Smith, Erwin F.: "Bacillus phytophthorus, Appel," Science, 1910, 31, p. 748.

Cohn's solution; potato starch not much acted upon; reduces nitrates; streaked on sterile raw potato from agar there is prompt growth along the line of the needle (twenty-four hours or less), with a brown stain and rapid disintegration and decay of the tissues. All varieties of potato are subject to the disease, but some to a much greater extent than others.

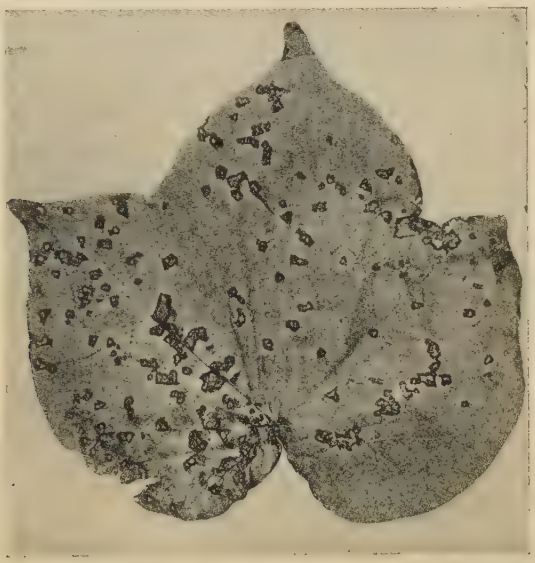


Fig. 184.—Angular leaf spot of cotton in which stomatal infections appear to be the rule. This leaf represents the secondary stage of a natural infection, *i. e.*, the spots are browned and shriveled and they involve the entire thickness of the leaf. In an earlier stage of the disease the spots are limited to the under side of the leaf (mesophyll), and occur in the form of small water-soaked areas surrounding the stomata, under which nests of bacteria occur. These spots gradually deepen so as to involve the palisade tissue, and then they become visible on the upper surface of the leaf. The spots are not yet shriveled or browned, but if the leaf is held up and viewed by transmitted light, they appear as translucent areas, while by reflected light they are dull and wet looking. A little later they present the appearance shown in this figure. All stages of this disease have been obtained by spraying upon the plants young agar cultures of Bacterium malvacearum suspended in sterile water (Erwin F. Smith).

The Black Rot of Cabbage and Allied Plants (Bacillus campestris).—The chief diagnostic character of this disease is the blackening of the fibrovascular bundles, which may be readily observed in the stem and leaf-stalks. There is an accompanying decrease in the water-flow, and the lower leaves of the cabbage wilt, turn brown, and drop off. Rutabagas and turnips are also attacked by the same disease, but not so seriously as are some of the other

members of the cabbage family. The micro-organism of the cabbage rot was first discovered by Pammel[1] in diseased Swedish turnips or rutabagas, and it has been more fully studied and its etiologic relations to the disease of cabbage and cauliflower established by Russell[2] and by Smith.[3] The disease is widely spread throughout the United States and is also common in Europe.[4] Infection of the plant may take place from the soil when the roots are injured at the time of transplanting, exposing the ends of the fibrovascular bundles, or it may occur from the bite of insects. There is evidence also that a common, perhaps the usual, mode of infection is through the water-pores at the margin of the leaves. The fluid which is exuded from these pores under favorable atmospheric conditions has been shown to be an excellent medium for bacterial growth, and the pores offer a ready means of ingress. The fluid may become infected by the visits of slugs or various insects or may be seeded with bacilli borne to the leaf by air-currents. The precise methods of dissemination from plant to plant have not been fully determined. In other bacterial infections the bacteria appear to enter the leaf through the stomata (Fig. 184).

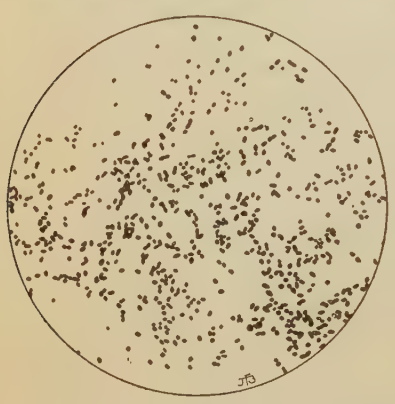


Fig. 185.—Bacillus campestris. Cover-glass (smear) preparation from the vessels of a cabbage plant received from Racine, Wis. Stained with carbol-fuchsin. Drawn from a microphotograph. × 1000 (Erwin F. Smith).

The bacillus of black rot, B. campestris, is a short, motile, gram-negative bacillus with rounded ends (Fig. 185). It grows readily in the ordinary culture-media, the optimum temperature being about 25° to 30° C. Gelatin is slowly liquefied and the casein in milk is gradually digested. In solutions of the various carbohydrates that have been tested no acid is formed. Upon potato a characteristic yellow pigment, perhaps a lipochrome, is produced, which is at first of a rather light color, but becomes darker with

[1] Pammel: Bull. No. 27, Iowa Agr. Expt. Sta., 1895, p. 130.
[2] Russell: Bull. No. 65, Wisc. Agr. Expt. Sta., 1898.
[3] Erwin F. Smith: Centralbl. f. Bakt., II, 1897, 3, p. 284.
[4] Harding: Centralbl. f. Bakt., II, 1900, 6, p. 305.

age. Inoculations of cabbages, cauliflowers, turnips, and other plants with pure cultures of B. campestris have reproduced the typical and characteristic disease (Fig. 186). Part of the injury done to the turnip root consists in the destruction of the cell wall, which is probably brought about by the solvent action of a cytolytic enzyme.[1] There is prompt action on potato starch.

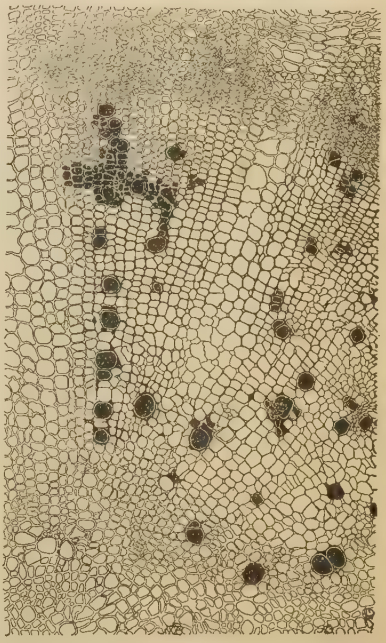


Fig. 186.—Cross-section of a turnip root, showing vessels occupied by Bacillus campestris as the result of a pure culture inoculation by means of needle pricks on the leaves. The bacteria are confined to the vessels and their immediate vicinity. They do not occur in the phloem, a small portion of which is shown at the top of the picture. Drawn from a microphotograph. × 57 (Erwin F. Smith).

Stem Blight of Alfalfa.—Sackett[2] has described a bacterial disease of alfalfa, widely distributed in Colorado and occurring in some other western states. In one district the first cutting of the alfalfa crop is said to have been reduced to one-fifth its original tonnage by the ravages of this malady. In the early stages a clear, yellowish, viscid liquid oozes from the diseased tissues and dries with a varnish-like luster on the stem. The leaves also usually show the disease, sometimes independently of the stem. "One-year-old plants may exhibit blackened areas in the crown, and black streaks which run down into the tap root. As the plant grows older, this blackening increases until the whole crown becomes involved, and either the crown buds are destroyed or the root is no longer able to perform its functions, and the plant dies."

A short, motile, aërobic bacillus (Bacillus medicaginis) has been isolated by Sackett from the diseased tissue. It grows readily on

[1] Erwin F. Smith: Bull. No. 25, Bureau of Plant Industry, U. S. Dept. of Agr., 1903.

[2] Sackett: Bull. 158, Colorado Agr. Exp. Sta., 1910.

the ordinary media at 28° C. It produces a fluorescent pigment on agar, does not liquefy gelatin, peptonize casein, or acidify milk. Inoculation of healthy plants with pure cultures produces the typical disease in seven to nine days.

The Yellow Disease of Hyacinths (Bacillus hyacinthi).—A disease of hyacinths studied by Wakker[1] and by Smith[2] is due to a bacillus closely related to B. campestris. The disease is manifested to the naked eye by a yellow striping of the leaves, which appears, as a rule, in long narrow areas separated by tracts of green

Fig. 187.—Bacterium stewarti filling the substomatic chamber and pushing out into the deeper tissues of a maize leaf. The result of an inoculation made by placing a small quantity of a pure culture on the tip of a sweet-corn leaf in the seedling stage. The globose bodies are nuclei (Erwin F. Smith).

tissue. The infection spreads to the bulb, which becomes filled with a bright yellow slime. Multiplication of the bacillus takes place chiefly in the vascular system; the walls of the vessels are destroyed, and large cavities formed in fibrovascular bundles.

B. hyacinthi is a small, motile, chromogenic bacillus which grows well on the ordinary culture-media. Gelatin and blood-serum are slowly liquefied. Yellow pigment is produced on most media and in the tissues of the host plant. Acid but no gas is formed in dextrose and saccharose broth. Milk is rendered alka-

[1] Wakker: Bot. Centralbl., 1883, 14, p. 315; Arch. neérland. d. sc. ex. et nat., 1889, 23, p. 1.

[2] Erwin Smith: Bull. No. 26, Div. of Veg. Physiol. and Path., U. S. Dept. of Agr., 1901.

line and the casein is precipitated. Indol is formed in peptone solution. The optimum temperature is about 28° to 30° C.; growth does not occur at 37° C. There is very slight action on potato starch.

According to Erwin F. Smith, B. hyacinthi, B. campestris, B. phaseoli (parasitic on beans; see below), and B. stewarti (parasitic on corn, especially sweet corn; Fig. 187) constitute a natural group. They are all bacilli with a single polar flagellum. All produce a yellow or brown pigment and live parasitically or semiparasitically on various plants. Cultural characters show a resemblance in many important particulars.

Citrus Canker.—A disease attacking various citrus trees, especially grape-fruit, seems to have been introduced into this country about 1912, probably on nursery stock from Japan. The causal organism was isolated by Clara Hasse,[1] and is described as an aërobic bacillus (B. citri) with a yellow growth on culture-media. Gelatin is liquefied. No gas is formed from carbohydrates. Inoculation of cultures on grape-fruit plants produces characteristic cankers.

This species is evidently very closely related to the bacillus causing a bean disease, next considered.

Bacterial Blight of Beans.—An important bacterial disease attacking the foliage, stems, and pods of the common beans and also the lima varieties is caused by a short motile rod with rounded ends (B. phaseoli). This organism produces a yellow growth on culture-media. Gelatin is liquefied and milk rendered slowly alkaline. On the pods and leaves appear small reddish spots, which increase rapidly in size and "develop into watery amber-colored blisters surrounded by a pink or reddish border. These blisters are filled with myriads of bacteria, and in time they dry down, forming a pale yellow or amber-colored crust over the affected areas. Ultimately the diseased leaves become brittle, ragged, and are worthless, while the pods curl, shrivel, and rot" (Sackett). It is supposed that the disease is introduced into a bean field by the seed and spreads from plant to plant by rain and leaf-eating insects.

Olive-knot (Bact. savastanoi = Bacillus oleæ in part).—Savastano in 1886–1887 found a cultivable bacillus constantly

[1] Hasse, Clara H.: Jour. Agric. Res., 1915, 4, p. 97.

present in the interior of young, growing olive galls.[1] Later he succeeded in infecting healthy plants with pure cultures of the bacilius and in practically establishing the etiologic relation of the micro-organism to the disease. The olive-knot is present on the trees of certain olive groves in California, where it seems to be due to the same bacillus described by Savastano. From this source it has been especially studied by Smith, who has cleared away much of the confusion caused by the use of mixed cultures

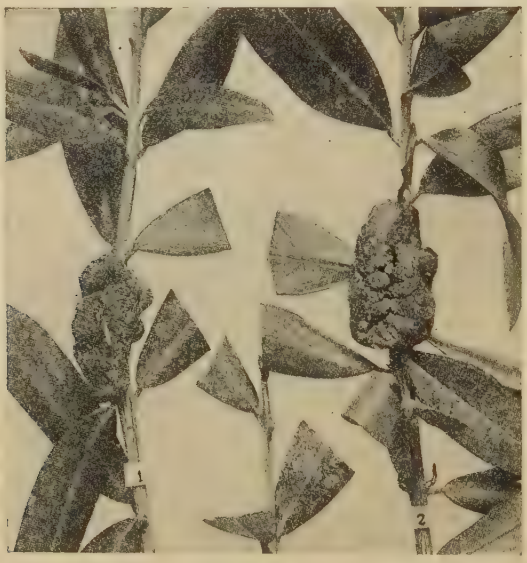


Fig. 188.—Bacterial olive-knots produced by needle-pricks of a pure culture. Inoculated January 4th; photographed May 16th. The organism came originally from an olive-knot obtained in California, where the disease has been very destructive (Erwin F. Smith).

and organisms of mistaken identity by other observers.[2] Smith's work has been thoroughly controlled by inoculation experiments (Fig. 188). The genuine olive-tubercle organism produces a whitish growth on various culture-media, does not liquefy gelatin, ferments dextrose but not lactose, and produces abundant alkali in litmus milk. It is actively motile, strongly anaërobic, and forms no spores. The interesting fact that tumors can occur by metastasis was discovered by Schiff-Giorgini, and has been confirmed experimentally by Smith, using pure cultures. The primary tubercles, which are

[1] Savastano: Compt. rend. de l'Acad. d. sc., 1886, 103, p. 1144.

[2] Smith, Erwin F.: Bull. No. 131, Bureau of Plant Industry, U. S. Dept. of Agri., 1908.

due to external infection, begin in the cortex. From the point of infection the bacteria make their way to distant points by way of the vascular system. The secondary tumors begin deep in the tissues at the junction of wood and pith.

FIG. 189.

FIG. 190.

Fig. 189.—Soft gall of peach producing hard gall on apple. Time, 2 years (Smith, Brown, and Townsend in Bulletin No. 213, Bureau of Plant Industry U. S. Dept. of Agriculture, 1911.)

Fig. 190.—Radial section through a daisy petiole, showing the internal origin of a small metastatic tumor. The normal tissues are bracketed, the epidermis is not yet ruptured, and the tumor includes all kinds of tissues peculiar to the petiole. (Smith, Brown, and Townsend in Bulletin No. 213, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1911.)

The Crown-gall of Plants.—A very common disease of plants occurs in the form of a swelling or gall of the crown, the junction of stock and scion just below the ground line (Fig. 189). It is especially likely to follow injury in grafting or cultivating. Smith, Brown, and Townsend[1] have described under the name Bacillus tumefaciens an organism first found by them in the galls of a cultivated marguer-

[1] Smith, Brown, and Townsend: Bull. 213, Bureau of Plant Ind., U. S. Dept. of Agri., 1911.

ite or daisy in 1904 (Fig. 190). Obtained in pure culture it produces typical galls when inoculated into healthy daisies. Cross-inoculations have shown that the same bacillus is capable of inducing tumors in widely separated species, genera, and families. The olive is not infected. The organism has also been isolated from natural galls on such plants as the willow, turnip, beet, hop, grape, poplar, cotton, rose, peach, and apple. Hard and soft galls and the "hairy-root" of apple seem to be etiologically similar.

The organism producing the plant tumor is a short, motile bacillus with polar flagella. It grows quite readily in the ordinary media, at room temperature forming white colonies on agar and gelatin plates. It does not liquefy gelatin, renders milk alkaline, and precipitates the casein.

These plant galls or tumors were believed by Smith to resemble malignant animal tumors in various particulars, such as their permanent and very rapid new growth, involving all the tissues of the part attacked; enormous round-celled or spindle-celled hyperplasia; great reduction of amount of conductive tissues; early necrosis, especially of the more fleshy tumors, with renewed growth at the margins; frequent recurrence after extirpation; extension of the disease to other parts by metastases. The disease progresses slowly, first stunting the plant and finally destroying it, unless removed by extirpation or by the development of increased resistance on the part of the plant.

Mode of Entrance and Spread.—In the preceding pages many instances have been given of the frequent conveyance of bacterial plant diseases by biting insects. The question whether bacteria can penetrate plant tissues only through wounds due to insect injuries or some other kind of crushing or bruising, or whether they can enter through natural openings, has been the object of considerable discussion. The fact that certain leaf-spot and fruit-spot bacterial diseases appear to spread with particular rapidity during rainy seasons might be thought to favor the view that under some conditions pathogenic bacteria can enter the uninjured plant through ordinary stomata. This assumption has been fully confirmed by experiment in several affections, such as the bacterial spot of peach and plum, the sweet corn disease due to Bacillus stewarti, the leaf-spot of cotton (Fig. 184), and Burrill's bacterial disease of broom-corn. Simply spraying pure cultures upon the

surface of leaves or fruit is sufficient to produce the disease in these instances.

Within the plant itself the bacteria seem to be able to pass from one part to another through vessels, through the parenchymatic tissues by the way of the intercellular spaces, and directly from cell to cell. Destruction of tissue or dissolution of cell-walls does not seem necessary.

Other Diseases.—Among other carefully studied bacterial diseases of plants may be mentioned a disease of cauliflower and

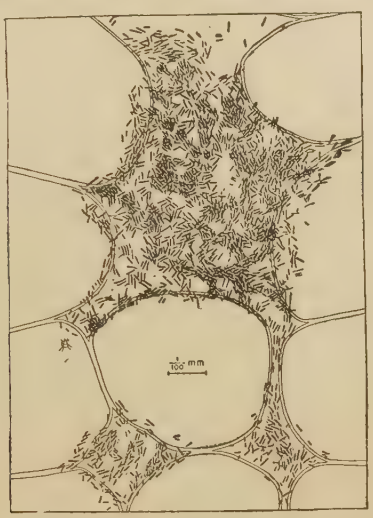


Fig. 191.—B. carotovorus wedging apart cells of the carrot. Drawn mostly from one plane. In placing the cover-glass a few of the bacteria have been crowded out of the intercellular spaces into parts they did not orginally occupy. × 500 (Erwin F. Smith).

allied plants described by Harrison (Bacillus oleraceæ[1]), a soft rot of carrots (Bacillus carotovorus[2]) (Fig. 191), a leaf-spot disease of broom-corn (Burrill), the so-called "gum disease" of the sugar-cane (Cobb), and a soft rot of the calla lily.[3] The so-called "bacteriosis of carnations" seems not to be a bacterial disease.[4]

Harrison[5] has made the interesting observation that different varieties of turnips show varying degrees of susceptibility to

[1] Harrison: Centralbl. f. Bakt., II, 1904, 13, p. 46.
[2] Jones: Centralbl. f. Bakt., II, 1901, 7, pp. 12, 61; also 1905, 14, p. 257.
[3] Townsend: Bull. 60, Bureau of Plant Ind., U. S. Dept. Agr., 1904.
[4] Woods: Centralbl. f. Bakt., II, 1897, 3, p. 722.
[5] Harrison: Centralbl. f. Bakt., II, 1904, 13, p. 197.

infection with Bacillus oleraceæ, the amount of rot present varying from less than 5 per cent. in some varieties to 65 per cent. in others. One variety seemed to be immune under natural conditions. A similar difference in susceptibility to bacterial infection has been observed in other plant diseases. Different varieties of peas, pears, cherries, and alfalfa show varying degrees of resistance to the attacks of pathogenic bacteria. It is not known upon what this varietal immunity depends.

APPENDIX

INFECTIOUS DISEASES OF DOUBTFUL OR UNKNOWN CAUSATION

THERE are a number of diseases that are infectious, and therefore in all probability due to some micro-organism, but concerning the actual causation of which there is at present either much difference of opinion among competent observers or absolute uncertainty.

HODGKIN'S DISEASE

A pleomorphic organism resembling the diphtheria bacillus has been found with considerable frequency in the lymphatic glands in the affection known as Hodgkin's disease. An etiologic relationship has been suggested by the work of some investigators.[1] Other studies on the bacterial flora of lymphatic glands[2] show that a variety of non-pathogenic bacteria can be cultivated from lymphatic glands, especially in diseased conditions. Further research in this field may give more definite results.

TRACHOMA

The name "trachoma" is given to an inflammation of the conjunctiva accompanied by the formation of so-called granules and, usually, of scar tissue. There is no absolute agreement, however, as to the symptoms and lesions of "true" trachoma, and the name has probably been applied to eye affections etiologically distinct. Trachoma is generally considered an infectious disease spread by contact, but there is much uncertainty with respect to the causal agent or agents.[3]

The disease is characteristic of poverty, uncleanliness, and generally unhygienic conditions of life. It appears to be easily controlled by the application of the rules of public and personal hygiene.

[1] Bunting and Yates: Jour. Amer. Med. Assoc., 1913, 61, p. 1803; 1914, 62, p. 516; Bull. Johns Hopkins Hosp., 1915, 26, p. 376.

[2] Bloomfield: Arch. Int. Med., 1915, 16, p. 197.

[3] See for example Royer: Jour. Am. Med. Assoc., 1926, 87, p. 482.

Cell inclusions have been found in the conjunctival epithelial cells in cases diagnosed as trachoma and have been regarded by many writers as bearing an important relation to the disease. They have been variously interpreted as being organisms akin to the protozoa ("Chlamydozoa" or mantle animals—Prowazek) and as "nests" of growing bacteria (Williams). Hemoglobinophilic bacteria similar to the influenza bacillus were found by Williams and her collaborators[1] in a large proportion of cases of chronic conjunctivitis and also in cases with trachoma inclusions. "These bacilli show morphologic and staining characteristics similar to those seen in trachoma inclusions; and transition forms between bacilli and inclusions are frequently seen in these cases" (Williams).

Noguchi[2] has been able to induce chronic granular conjunctivitis in monkeys by inoculation of pure cultures of a micro-organism isolated from cases of trachoma in American Indians.

Colds

Little is definitely known about the bacteria causing the common colds Undoubtedly, the term "cold" is used for a variety of affections etiologically distinct. Pneumococci and streptococci, as well as the influenza bacillus and even the tubercle bacillus, may be responsible for the train of symptoms to which the name "cold" is popularly applied. Colds are apparently largely spread by close personal contact and by the infectious droplets discharged in sneezing and coughing. Whether certain micro-organisms are more commonly concerned in the production of colds than others is quite uncertain. Some specific organisms have been implicated. An anaërobic organism has been found in some series of cases of acute and chronic rhinitis and has given rise to rhinitis when introduced experimentally into the nose of a healthy person.[3] Specific antibodies are present in the sera of persons infected experimentally and of those suffering from a natural infection with this bacillus. A specific filterable virus has been reported by some observers (Foster).

[1] Anna W. Williams *et al.*: Jour. Infect. Dis., 1914, 14, p. 261. This article contains a comprehensive survey of the subject.

[2] Noguchi: Bull. N. Y. Acad. of Medicine, 2nd series, 1927, 3, p. 295.

[3] Ruth Tunnicliff: Jour. Infect. Dis., 1913, 13, p. 283; 1915, 16, p. 493.

Mumps

A diplococcus was found in the exudate of the parotid gland by Laveran and Catrin[1] in a large proportion of all cases examined. Other investigators have found a similar diplococcus in the gland exudate and in the blood in cases of mumps. Korentschewsky[2] obtained a diplococcus from the exudate in twenty-one out of twenty-nine, and from the blood in eight out of thirty-two cases. A diplococcus isolated by Isabella Herb[3] shows the following characteristics: It averages from 0.5 to 1.5 μ in diameter, stains easily with the ordinary anilin dyes, and is gram-positive. It has no capsule. Development takes place on all the ordinary media, but is very slow, the colonies on glycerol-agar being scarcely visible in twenty-four hours. Gelatin is very slowly liquefied. Milk is coagulated in forty-eight hours. Mixing sterilized saliva with agar, as in making blood-agar, gives a favorable medium, and in twelve to twenty-four hours an abundant growth occurs. This diplococcus apparently corresponds with the organism isolated by Laveran and Catrin. Miss Herb's inoculation experiments are significant. "Inoculations of suspensions of the diplococcus into Steno's duct in the monkey and in the dog produce an acute, uniform enlargement of the parotid gland, accompanied with some slight fever. In the dog this enlargement is the result of an infiltration that consists largely of mononuclear cells, and is accompanied with a general increase in the mononuclear cells in the blood, as well as a distinct rise in the opsonic index with respect to the diplococcus."

Other observers have not been able to confirm these findings, but have reported finding a filterable virus, Wollstein[4] succeeding in producing parotitis and orchitis in cats by inoculations of filtered saliva from cases of mumps.

Sprue

This is a tropical disease characterized by a catarrhal inflammation of the alimentary tract. The mouth and tongue show a peculiar ulcerative condition. The stools are pale and frothy. Anemia and general wasting occur. Ashford reports that in Porto Rico it is

[1] Laveran and Catrin: Compt. rend. de la Soc. Biol., 1893, 45, p. 95
[2] Korentschewsky: Centralbl. f. Bakt., I, Orig., 1907, 44, p. 394.
[3] Herb, Isabella: Archives of Int. Med., 1909, 4, p. 201.
[4] M. Wollstein: Jour. Exper. Med., 1916, 23, p. 353.

far more common and fatal among American residents than tuberculosis.

A yeast-like organism has been observed in the stools of sprue patients by a number of investigators, and its relationship to the disease has been especially emphasized by Ashford.[1] The yeast in question, named by Ashford Monilia psilosis, is found by him to be constantly present in the mouths and in the feces of patients suffering from sprue. Serological tests, including the complement-fixation reactions, support the view of a causal relation. Experiments by L. W. Smith[2] are confirmatory of Ashford's conclusions. It is shown that gastro-intestinal lesions roughly comparable to those found in man may be produced in the guinea-pig by intraperitoneal and oral inoculation with Monilia psilosis. Lesions rarely develop, however, except in animals kept on a deficiency diet. These experiments give strong support to the view that sprue originates as a food deficiency disease and that Monilia—or some other fungus of relatively low pathogenicity—becomes implanted in the weakened organism.

Influenza

Epidemic influenza, so far as can be recognized by the meager historical descriptions, occurred at least as early as the fourteenth century. Since the beginning of the sixteenth century epidemic waves of this disease have swept over the world at more or less frequent intervals, the latest being the great pandemic of 1918–19. The loss of life in the recent outbreak was enormous, amounting to over 450,000 deaths in the United States and to over 6,000,000 in India. Among the characteristic features of influenza epidemics are: the great morbidity—from 25 to 50 per cent. of the population or higher; the rapid spread of the disease; the age incidence—young adults of the ages twenty to thirty being especially affected; and the tendency to the development of pneumonia and other complications.

In the years between pandemics, for example between 1890 and 1918, many deaths have been attributed to "influenza," but since recognition of true influenza in non-epidemic periods is hardly possible by either clinical or bacterial methods the identity of the

[1] B. K. Ashford: Amer. Jour. Med. Sci., 1915, 150, p. 680; 1917, 154, p. 157.

[2] L. W. Smith; Jour. Amer. Med. Assoc., 1924, 83, p. 1549.

"influenza" of 1917 with the epidemic influenza of 1918 is uncertain. It is perhaps significant that the age-grouping of the "influenza" deaths in non-epidemic periods is very different from that in pandemics.

The mode of transmission of influenza is not known. The conspicuous symptoms of respiratory involvement in many cases have naturally led to the supposition that the infecting agent is present in the discharges from the nose or throat. Experiments upon human volunteers, however, made by spraying such discharges upon the mucous surfaces of healthy persons or by direct exposure to coughing and sneezing influenza patients in various stages of the disease, have been singularly unsuccessful in producing infection. Leake[1] obtained results that could be regarded as positive in only a very small proportion of cases in spite of the drastic methods used to produce infection. This is in contrast to the rapidity with which the disease spreads under natural conditions.

If immunity is conferred at all by an attack of influenza it seems to be very transient.[2]

The specific organism that has been most commonly regarded as the cause of influenza since the pandemic of 1890 is the hemophilic bacillus discovered by Pfeiffer (Hemophilus influenzæ, p. 374). Pfeiffer's bacillus, although not found by Pfeiffer until 1892, after the initial wave of true influenza had passed, was generally regarded by bacteriologists up to 1918 as the causal agent of influenza. There were some dissenting voices and these have swelled into a chorus of negation as a consequence of the bacterial observations made in 1918–20. The chief reasons for doubting that Pfeiffer's bacillus is the cause of influenza are these: (1) the finding of this organism—or one not at present distinguishable from it—in the throats of healthy persons and of persons suffering from other diseases, such as measles, tuberculosis, and whooping-cough; (2) its occurrence in miscellaneous respiratory infections during non-epidemic periods with nearly if not quite as great frequency as in influenza epidemics; (3) its relative abundance in certain localities in the epidemic period and its relative scarcity in others—this appears to be demonstrated by the postmortem examinations in the U. S. army camps even when allowance is made for

[1] Leake: Boston Med. and Surg. Jour., 1919, 181, p. 675.
[2] Jordan and Sharp: Jour. Infect. Dis., 1920, 26, p. 463.

differences in technic of isolation; (4) the signal failure of most inoculation experiments. Rosenau[1] describes experiments made upon 19 volunteers each of whom was given "a very large quantity of a mixture of thirteen different strains of the Pfeiffer bacillus, some of these obtained recently from the lungs at necropsy." Suspensions of these organisms were sprayed with an atomizer into the nose and into the eyes, and back into the throat, several billions of the bacteria being introduced in this way into the respiratory tract of each volunteer, but no illness resulted; (5) the fact that in the rare cases when infection of any kind has been produced in man by the inoculation of Pfeiffer's bacillus it has not borne any close clinical resemblance to influenza[2]; (6) the failure of recently effected inoculation with the Pfeiffer bacillus to protect against attacks of influenza.

On the other hand, the great frequency with which the Pfeiffer bacillus is found in all parts of the world in the pneumonic lesions associated with influenza, and the important experimental results simulating the production of true influenza obtained by Blake and Cecil[3] through monkey inoculations (cf. p. 378) must be taken into consideration in any discussion of the relation of this organism to influenza. For the present it seems possible only to return the verdict "not proved."

A great variety of other micro-organisms are found associated with cases of influenza both in the upper respiratory tract during the attack and in the pathologic lesions after death. Among these are streptococci, especially of the green-producing type, pneumococci, staphylococci, N. catarrhalis, Friedländer's bacillus, and various nondescript organisms. In some instances these organisms, as in the case of certain green-producing streptococci, possess an exceptionally high virulence and seem to be a potent factor, at least in the late stages of the influenza processes; their absence from certain localities and from certain cases and epidemics of influenza is, however, an obstacle to accepting them as the primary cause. Perhaps the most plausible explanation of these bacteriological findings is to regard the streptococci, N. catarrhalis forms and the like (including Pfeiffer's bacillus?), as secondary invaders whose

[1] Rosenau: Jour. Amer. Med. Assoc., 1919, 73, p. 11.
[2] Davis: Jour. Infect. Dis., 1906, 3, p. 1.
[3] Blake and Cecil: Jour. Exper. Med., 1920, 32, pp. 691, 719.

pathogenicity becomes heightened in the presence of the true causal agent of influenza.

The manifestly unsatisfactory nature of the evidence with respect to the ordinary cultivable bacteria has led many investigators to make search for a filterable virus. Some of the earlier attempts in this direction were not made with suitable technical methods and the results have not stood destructive criticism. The most promising work in this field is that of Olitsky and Gates,[1] who recovered from filtrates of nasopharyngeal washings of epidemic-influenza patients a minute, bacilloid body to which they gave the name of Bacterium pneumosintes (lung-devastator). It is quite uniform in shape and size, strictly anaërobic, gram-negative, staining with difficulty with the usual basic dyes. It passes Berkefeld filters *V* and *N*; resists freezing and drying, but is killed by a temperature of 56° C., and withstands the action of glycerol for many months.

Rabbits inoculated with nasopharyngeal washings or pure cultures of Bact. pneumosintes develop leukopenia and hemorrhagic areas in the lungs similar to those observed in human influenza. Bact. pneumosintes is demonstrable in the lung tissue of inoculated rabbits.

Identical antigenic characters were manifested by ten different strains of Bact. pneumosintes from the influenza epidemics of 1918, 1920 and 1922. Resistance to intratracheal inoculation was developed in rabbits injected with killed cultures of this organism, while control animals suffered typical infections.

The inoculation of human volunteers with Bact. pneumosintes led to transitory leukocytosis and the appearance of specific agglutinins in the blood. Agglutinins were demonstrated in the serum of 17 out of 19 persons examined within five months after recovery from epidemic influenza. In most of the cases where agglutinins occurred, specific precipitins also were found.

Epidemic Encephalitis

An affection of the central nervous system which came first into special prominence soon after the influenza epidemic of 1918–19, although an outbreak had been observed in Vienna in the winter of

[1] Olitsky and Gates: Jour. Amer. Med. Assoc., 1920, 74, p. 1497; 1921, 76, p. 640; Jour. Exper. Med., 1921, 33, pp. 125, 361, 373, 713; 1921, 34, p. 1; 1922, 35, pp. 1, 553, 813; 1922, 36, pp. 501, 685; 1923, 37, pp. 303, 471.

1916, is now generally recognized as an independent disease. The common occurrence of marked somnolence has given it the popular and confusing name of "sleeping sickness.[1]" Evidence can sometimes, although rarely, be obtained of contact with a previous case.

Attempts to induce the disease in animals have met with difficulty and the results are conflicting. Monkeys are apparently refractory. Rabbits are infected readily according to some observers, very uncertainly according to others. Flexner,[2] in the course of a four years' investigation, during which the total number of cerebrospinal fluids from all sources injected exceeded a hundred, obtained but a single instance interpretable as a successful infection. Reported experiments offer some perplexing features. The virus found in the common herpetic vesicles on the lips (cold sores) may easily be inoculated upon the cornea of rabbits, and it has been found that the virus of herpes and the supposed virus of encephalitis obtained by some investigators bear a close relationship one to another, and can indeed not be distinguished. A special difficulty in this field is the common occurrence of brain lesions in uninoculated laboratory rabbits, so that great caution is necessary in interpreting the consequences of specific injections.

Loewe and Strauss[3] have reported cultivating from affected tissues minute globoid bodies which they regard as the specific organisms of the disease. Levaditi and other experimenters obtained negative results in their attempts to grow a micro-organism from the virus. Several investigators[4] have cultivated a pleomorphic streptococcus which seems to have specific characters and is perhaps the causal organism of this disease. The organism is highly virulent for rabbits and when inoculated intravenously is said to show a tendency to elective localization in the brain. The symptoms in rabbits and monkeys are said in some instances to simulate those of human epidemic encephalitis.

Encephalitis has been regarded by some as a direct sequel of influenzal infection, or as due to a highly neurotrophic influenzal

[1] This disease has, of course, no relation to the African sleeping sickness caused by a Trypanosoma (p. 569).

[2] Flexner: Jour. Amer. Med. Assoc., 1923, 81, p. 1785.

[3] Loewe and Strauss: Jour. Amer. Med. Assoc., 1919, 73, p. 1056; Jour. Infect. Dis., 1920, 27, p. 250.

[4] Von Wiesner: Wien. klin. Wchnschr., 1917, 30, p. 933; Rosenow: Jour. Infect. Dis., 1924, 34, p. 329; Evans and Freeman: Public Health Reports, 1926, 41[1], p. 1095; Evans: Public Health Reports, 1927, 42[1], p. 171.

virus, but there are grave objections to these views. It has also been suggested that the encephalitis virus is widespread in normal persons, but needs "activation" by the influenza virus. The existence of any relation whatever between influenza and encephalitis may, however, be questioned.

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